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Draining sinus and swelling in right hypochondrium; Actinomycosis

Courtesy: Dr. Samreen Sarfaraz, The Indus Hospital, Karachi.

MDR TB: Monster of our own Creation

“MDR TB is a man made problem.....It is costly, deadly, debilitating, and the biggest threat to our current TB control strategies.”

Pakistan has the dubious distinction of having the world's eighth largest burden of TB (WHO Report, 2008)¹. The numbers of multidrug resistant TB (MDR-TB) too have grown exponentially. MDR is a laboratory diagnosis, defined as *in vitro* resistance to the two most effective first line drugs: isoniazid (H), and rifampicin (R), while an even more sinister form of drug resistance –Extensive Drug Resistance (XDR) is defined as *in vitro* resistance to H,R, plus any one of the aminoglycosides plus any one of the fluoroquinolones. There are several reports of MDR-TB rate of 2.4% in new patients, and 13.9% in previously treated patients in Pakistan²⁻⁵. Conditions that select for MDR include: high bacillary load, low drug levels and exposure to multiple previous regimens. The most consistent contributory factor of all is inadequate treatment. Wrong prescription writing by health care providers has been a major contributor. Non adherence because of poor communication with the health care provider, complex regimens, unattended side effects, discontinuation by the patient after initial improvement or unaffordability of medicines cause the patient to default.

Delay in diagnosis and treatment leads to lung cavities which can harbor as many as 10⁸ rapidly dividing bacilli. The drug resistant strain can be transmitted to close contacts through aerosolization. Even after treatment these cavities may result in parenchymal damage, thus severely and permanently compromising lung function. Incorrect or missed diagnosis is often blamed on poor sputum production and inadequate testing facilities. This is being largely improved by rapid point of care testing through molecular identification of *Mycobacterium tuberculosis* (MTb). WHO has approved the GeneXpert, which is expected to revolutionize early detection of MTb as well as

rifampicin resistance. If cases can be rapidly and correctly detected, early and correct treatment would reduce complications with prevention of drug resistance⁹.

It therefore behooves the treating doctor to diagnose pulmonary TB as early as possible by testing sputum for acid fast bacilli, and become proficient at treating TB appropriately. Training courses beginning in medical colleges would provide the ground work for good medical care. A combination of antibiotics calculated on basis of the patient's weight for 6 to 8 months, and at least monthly clinical and bacteriologic follow up will not only serve to cure the patient and reduce risk of transmission but also minimize chances of emergence of MDR.

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Etiological Factors causing Persistence of Symptoms in Treated Patients of Pulmonary Tuberculosis

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Abstract

Objective

To find out etiological factors causing persistence of symptoms in treated patients of pulmonary tuberculosis.

Methods

The study was carried out in the Department of Pulmonology, Military Hospital Rawalpindi. Diagnosed patients of tuberculosis having received at least six months of anti-tuberculosis treatment and presenting with persistence of symptoms i.e. weight loss, hemoptysis, productive cough and fever were included in the study. CT scan of the patients was done followed by fiberoptic bronchoscopy. Bronchial washings of each patient were taken and submitted for acid fast bacilli smear, *Mycobacterium tuberculosis* culture and sensitivity, bacterial and fungal cultures.

Results

Ninety patients fulfilling the criteria were included in the study. Mean age patients was 42 years (± 17 ; ranged 15 to 80 years). 31 (35%) patients were males and 59 (65%) females. 37 (41%) patients showed no evidence of infection with any microbiological organism, 22 (24%) patients had bacterial infection and 8 (9%) patients fungal infection as detected by bacterial and fungal cultures respectively. Bronchial washings of 23 (25%) patients yielded growth of *Mycobacterium tuberculosis*. The Ziehl-Neelsen stained smears prepared from bronchial washings showed acid fast bacilli positivity in 15 (17%) of cases. CT scan revealed bronchiectasis in 34(38%) patients, cavitating lung lesion in 30(33%) patients and no significant structural abnormality in 26(29%) patients.

Conclusion

Patients of tuberculosis presenting with persistence of symptoms having received anti-tuberculosis treatment require a thorough diagnostic work up including radiological investigations to rule out secondary bacterial or fungal infections and drug resistance

so that further management can be done accordingly.

Key Words

Mycobacterium tuberculosis, Pulmonary Tuberculosis

Introduction

Pulmonary tuberculosis (TB) is a major health burden worldwide.^{1,2} Pakistan is one of the countries where the disease prevalence is very high.^{3,4} Most of the patients on standard anti-tuberculosis treatment (ATT) respond to treatment and show symptomatic improvement, sputum smear and culture conversion, however, a significant number of patients develop breakthrough symptoms or continue to experience symptoms even after prolonged treatment.^{5,6} Persistence of these symptoms has varied etiology like secondary bacterial infections, bronchiectasis or structural lung damage, multidrug resistant (MDR) TB and fungal infections.⁷⁻⁹ Searching for the etiology is quite exhaustive and requires a battery of investigations.

In the developing countries like Pakistan, diagnostic facilities are not available in most parts of the country and cost of investigations hinders the diagnostic work up, it becomes even more difficult. These patients are usually managed with antibiotic courses for presumed secondary bacterial infections or extended duration of ATT.¹⁰ Administration of unnecessary antibiotics or prolonged administration of ATT may lead to drug resistance. Also if the correct cause is not detected appropriate treatment cannot be given resulting in further persistence of symptoms which may lead to complications. We conducted this study to find out the etiology of persistent or breakthrough symptoms in treated patients of pulmonary tuberculosis. By working out the etiological factors in patients with persistent symptoms it would be easier to formulate a standard diagnostic work up for these patients.

Material and Methods

It was a descriptive study carried out in the Department of Pulmonology, Military Hospital Rawalpindi, from January 2009 through September 2010. Approval of the Institutional Ethical Committee was obtained. After detailed history and thorough

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physical examination, selection of patients was done by non-probability convenience. Both indoor and outdoor diagnosed patients of tuberculosis having received at least six months of anti-tuberculosis treatment, patients more than 12 years of age and with persistent symptoms of weight loss, hemoptysis, productive cough and fever were included in the study. Patients having extra pulmonary tuberculosis, cases of multi-drug resistant (MDR) TB on initial diagnosis and patients suffering from epilepsy, diabetes mellitus, renal failure, chronic liver disease and AIDS were not included in the study.

Written consent was taken from all the patients included in the study. Patients underwent x-ray chest and CT scan using 64 slice multi-detector CT (Toshiba Company); findings were noted. It was followed by fiberoptic bronchoscopy using flexible Olympus P-60 fiberoptic bronchoscope. Patients were pre-medicated with 2% xylocaine spray for local anaesthesia and thorough examination of tracheobronchial tree was performed and the bronchial washings were taken and submitted for *Mycobacterium tuberculosis* (MTB) culture and sensitivity, acid fast bacilli (AFB) smear, bacterial and fungal culture. For MTB culture and sensitivity, BACTEC 460 system using BACTEC 12B liquid medium was used and the samples were inoculated for detection of MTB growth. The samples showing growth of MTB were further inoculated for sensitivity testing of all anti-TB drugs. Smears were also prepared from bronchial washings on glass slides and these were then stained with Ziehl-Neelsen (ZN) stain. Samples were inoculated on standard bacterial culture media including blood agar (Oxoid, UK) and McConkey agar (Oxoid, UK) and incubated for 48 hours. For detection of fungal growth, Sabouraud's Dextrose agar (Oxoid, UK) with chloramphenicol was used.

The results of these tests were recorded and data collected was analyzed by using SPSS version 18.0. Frequencies and percentages of descriptive variables were calculated. Mean with standard deviation was calculated for numeric variables such as age.

Results

Ninety patients fulfilling the criteria were included in the study. Mean age of patients was 42 years (± 17) with range of 15 to 80 years. Thirty one (35%) patients were males and 59 (65%) were females.

37 (41%) patients showed no evidence of infection with any microorganism. 22 (24%) patients were having bacterial infection and 8 (9%) patients had fungal infection as detected by bacterial and fungal culture respectively. Bronchial washings of 23 (25%) patients yielded growth of MTB on culture. Out of 23 patients having persistent TB, the culture and sensitivity report revealed that only 8 patients were sensitive to first line ATT while the rest were resistant to one or more drugs (Table 1). Gender wise distribution of the findings of results is summarized in table 2.

Bronchial washing smears of 15 (65%) patients were positive

for AFB from 23 patients having MTB positive culture growth and 8 (35%) smears were negative. All 67 culture negative cases were also negative for AFB smear i.e. true negative and there were no false positive cases. The sensitivity and specificity of bronchial washings smear for AFB in this study was 65% and 100% respectively taking culture for MTB as gold standard.

Correlation between the results of MTB culture and AFB smear is summarized in table 3. Correlation of structural lung damage detected on CT scan and the presence of infectious agents is summarized in table 4.

CT scan revealed bronchiectasis in 34(38%) patients, cavitating lung lesion in 30(33%) patients and no significant structural abnormality in 26(29%) patients.

Discussion

Tuberculosis is a major health burden worldwide and it is a major health issue in Pakistan.¹ Pakistan ranks sixth among the high TB burden countries in the world.⁴ Approximately 420,000 new cases of TB occur every year and almost half of them are sputum smear positive for AFB. With such high incidence rate and increased burden on health care resources, the early detection

Table 1: Sensitivity and resistance pattern in patients having persistent TB (n=23)

Sr. #	Resistance Pattern	n(%age)
1.	Resistant to INH	4(18%)
2.	Sensitive to first line ATT drugs	8 (35%)
3.	Resistant to Rifampicin	1(4%)
4.	Resistant to INH and Rifampicin	1(4%)
5.	Resistant to INH, Rifampicin and Streptomycin	2(9%)
6.	Resistant to Ethambutol	2(9%)
7.	Resistant to INH, Rifampicin, Ethambutol and PZA	2(9%)
8.	Resistant to Rifampicin and Streptomycin	1(4%)
9.	Resistant to INH and Streptomycin	1(4%)
10.	Resistant to INH and Ethambutol	1(4%)

INH - Isoniazid

PZA - Pyrazinamide

Table 2: Gender wise distribution of isolates

Gender	Etiology				Total
	No growth	Fungal infection	Bacterial infection	Positive MTB culture	
Male	12	0	11	8	31
Female	25	8	11	15	59
Total	37	8	22	23	90

Table 3: Correlation of AFB smear and MTB culture

Bronchial washings smear for AFB	Bronchial washings culture for MTB		Total
	Positive for MTB growth	Negative for MTB growth	
Positive for AFB	15	0	15
Negative for AFB	8	67	75
Total	23	67	90

close monitoring.¹³ According to Al-Hajjaj, chest radiographs showed complete clearance of lesions in 43.5% of patients <20 years old and in 30.3% of patients > 40 years after being treated for TB.¹⁴ The cavitation was present in 18.3% and 11.9% in both groups respectively in the same study whereas in our study the clearance of lesion was seen in 29% and cavitation in 33% of patients. According to Denning *et al*, 25% of patients suffering from pulmonary TB in the US and 35% in Taiwan, China developed cavitation.¹⁵ In the same study, 22% of patients of pulmonary TB developed chronic pulmonary aspergillosis, 15% of TB patients developed aspergilloma in a study conducted by Byun *et al* compared to 9% of patients who developed fungal infection in our study.¹⁶

In a study conducted by Kim *et al*, 3.5% of total patients

Table 4: Correlation between CT findings and infectious agents

CT scan chest findings	Etiology				Total
	No growth	Fungal infection	Bacterial infection	Persistent TB	
Bronchiectasis	8	4	12	10	34
Cavitating lung lesions	6	4	10	10	30
No significant finding	23	0	0	3	26
Total	37	8	22	23	90

and treatment of TB has gained importance. The standard ATT has revolutionized the outcome of this disease and most patients respond to treatment and show symptomatic improvement with smear and culture conversion. There are, however, significant number of patients that continue to experience the symptoms of TB including weight loss, fever, productive cough and hemoptysis.⁵ There is varied etiology for persistence of these symptoms including multi-drug resistant TB, secondary bacterial infections or fungal infections.⁷⁻⁹ There can also be structural lung damage which can be secondary to the above mentioned infectious agents.

In our study, out of 23 patients having persistent TB, only 3 patients showed no significant findings on CT scan of chest. These results showed that there was a fairly accurate correlation of presence of any infectious agent with the structural changes detected on CT scan. Follow up CT scan is a good mean of predicting presence of any infectious etiology in patients of TB having persistent symptoms as well as assessing the efficacy of ATT and patients showing no significant findings on CT scan chest are less likely to have any bacterial, fungal or TB infection.^{11,12} CT scan findings can also predict the course of treatment and follow up; according to Bang, patients of TB showing cavitating lung lesions on CT scan have higher incidence of relapse and may require prolonged treatment and

suffering from TB experienced bacterial infection compared to 24% in this study.¹⁰ Given the fact that ATT also has anti-bacterial activity, there are more chances of emergence of multi-drug resistance (MDR) bacteria in treated patients of TB as evidenced by study of Kim *et al* where 48% of TB patients having bacterial infection were infected with MDR strains.¹⁰ It therefore becomes very important to rule out the presence of bacterial infection in patients of TB having persistent symptoms as the likely chances of MDR bacterial infections are high and require change in treatment protocol.

In the present study 26% were having MDR TB as compared to 38% (2008) and 39% (2009) in Pakistan according to Hasan *et al*, 9.5% by Byun *et al* and 13.8% in MTB positive cases of endobronchial washings by Ikram *et al*.¹⁶⁻¹⁸ The limitation in this study was less number of cases. In a study by Melzer *et al*, in 392 patients of culture proven TB, 33 (8.5%) patients were having drug resistant TB and of these 29 (87.9%) were isoniazid resistant, 3(9.1%) MDR, and 1(3.0%) was rifampicin resistant.¹⁹ In our study, 18% were isoniazid, 26% were MDR TB and 4% were rifampicin resistant. According to Ikram *et al*, 89 specimens including sputum and endobronchial washings were positive for MDR TB, and out of these 33 (37%) were two drug resistant, 12 (13%) were three drug resistant and 44 (50%) were four drug resistant.¹⁸

The sensitivity and specificity of bronchial washings smear for AFB in this study was 65% and 100% respectively taking culture for MTB as gold standard. When compared with other studies, the percentage of AFB positive patients on ZN staining of bronchial washings was 48.3, 42% and 50% according to Bachh *et al*, Ansari *et al* and Caymmi *et al* respectively.²⁰⁻²² Sputum or bronchial washing smears for AFB is a cheap, simple and rapid method for detection of AFB and although not all patients suffering TB are positive but keeping in view the high percentage of detection it can be used as one of the methods in diagnostic work and follow up of TB.

Conclusion

Patients of tuberculosis presenting with persistence of symptoms having received anti-tuberculosis treatment require a thorough diagnostic work up to rule out secondary bacterial or fungal infections and drug resistance so that further management can be done promptly and accordingly.

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Trend of Methicillin Resistance in Coagulase Negative Staphylococci Isolated from Blood Culture Specimens

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Abstract**Objective**

To determine the frequency of methicillin resistance among coagulase negative staphylococci isolated from blood culture specimens.

Methodology

This retrospective descriptive study was conducted at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi. Data was collected from the blood culture records of the department. All coagulase negative staphylococci isolated during the period from January 2007 to December 2010 were included and sensitivity to methicillin was noted.

Results

A total of 217 coagulase negative staphylococci were isolated from 12188 blood culture specimens received in the department. Out of these, 41% showed methicillin resistance during 2007, 59% during 2008, 60% during 2009 and 66 % during 2010.

Conclusion

Methicillin resistance is increasing among coagulase negative staphylococci, isolated from blood samples of patients in our set up.

Key Word

Blood Culture, Coagulase Negative Staphylococci, Methicillin Resistance,

Introduction

Coagulase-negative staphylococci (CoNS) are Gram positive, catalase positive bacteria which are part of normal flora of skin. Important species in this group include *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. capitis*, etc. Previously, CoNS isolated from the blood culture specimens of hospitalized patients were often considered as contaminants, but in the last one decade these have emerged as important nosocomial pathogen, especially with reference to blood stream infections.^{1,2} They are responsible for 30 % of all nosocomial blood stream infections. In addition, they can cause endocarditis, urinary tract infections, central nervous system shunt infections,

endophthalmitis, surgical site infections, peritonitis and foreign body infections. The most important aspect of treating CoNS related infections is the methicillin resistance. Studies reveal that 55-75 % of nosocomial isolates are methicillin resistant.³ Methicillin resistant isolates are usually resistant to most other antibiotics as well. Vancomycin, an expensive antimicrobial and administered parenterally, is usually the drug of choice for treatment of infections caused by these methicillin-resistant CoNS.⁴ There is no documented study from Pakistan to know the prevalence of methicillin resistance among CoNS isolated from the blood culture specimens. This study was conducted to know the trend of methicillin resistance among CoNS isolated in our institute.

Methodology

We conducted a retrospective study at the Microbiology department, Armed Forces Institute of Pathology Rawalpindi. All the blood culture results were considered for the presence of coagulase negative staphylococci in the last four years i.e. from Jan 2007 to Dec 2010. Presence of staphylococci was confirmed by colony morphology, Gram staining and catalase test. DNase agar was used to differentiate coagulase positive from CoNS. Cefoxitin disc (30µg) was used to check methicillin susceptibility. Mueller Hinton agar (Oxoid, UK) was used for cefoxitin susceptibility and culture plates were placed at 37°C for 12-18 hours. Zone of inhibition of more than or equal to 25 mm was considered as methicillin sensitive whereas less than or equal to 24 mm as methicillin resistant.⁵

Results

During the period of study, a total of 12188 specimens were received for blood culture. 1480 specimens yielded various isolates, out of which 217(14.7%) were CoNS. Out of these 217 isolates, 54 were yielded in 2007, 56 in 2008, 57 in 2009 and 50 in 2010. The frequency of year wise methicillin resistant CoNS is shown in table 1.

Discussion

Being part of normal flora of the skin, CoNS can contaminate the blood culture specimens. Although these are the most commonly isolated contaminants from blood cultures, yet they are the most frequent cause of true bloodstream infections as well.⁶ A study done in the United States to determine the incidence of significant CoNS bacteremia versus that of pseudobacteremia, in which a total of 3,276 cultures of blood

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Table 1: Methicillin susceptibility of isolated CoNS

Year	CoNS (n)	Methicillin resistant n(%)
2007	54	22(41%)
2008	56	33 (59%)
2009	57	34 (60%)
2010	50	33 (66%)

from 1,433 patients were evaluated, showed that 72% of the blood cultures had CoNS as contaminants but still 24% patients had CoNS bacteremia.⁷ Methicillin resistance among the CoNS makes it more difficult to treat such infections. Methicillin resistance is caused by *MecA* gene, which results in the production of an altered penicillin binding protein PBP2a.⁸ PBP2a does not allow the attachment of penicillinase-resistant penicillins to bind to it and so the bacteria escape from the effect of these antibiotics. CoNS are usually multidrug resistant, making their treatment even more difficult. Prior use of antibiotics and prolonged nursing home stays are few important causes of this increasing multidrug resistance trend among CoNS.⁹

The rising trend of methicillin resistance among CoNS, as revealed in our study is in concordance with other international studies. A study conducted in Denmark (1996) showed that 32% of all CoNS isolated were methicillin resistant.¹⁰ An Indian study conducted in 2004 showed that 66% of CoNS isolated from blood culture specimens were methicillin resistant.¹¹ Another study from Turkey (2006) showed that 92% CoNS were methicillin resistant.¹²

It is estimated that physicians use antimicrobial agents to treat nearly one-half of the patients with contaminated blood cultures; vancomycin being the most misused drug.⁶ This also results in manifold increase in the physical, psychological and financial sufferings for the patients. Contamination of blood culture specimens by CoNS can be prevented by following proper disinfection of the venipuncture site to avoid skin flora contamination.⁹ To decrease the rising trend of methicillin resistance among CoNS, clinicians should be advised to avoid unnecessary use of antibiotics, and hospital staff should be instructed to follow the patient care guidelines properly. Special care should be taken in making decisions for treating or otherwise of the contaminated blood culture specimens, especially in set

up like our where specimens are received from immune-compromised patients like oncology ward, and with bone marrow and renal transplant patients.

Our study had certain limitations; it was a laboratory based study and so correlation of the blood culture results with the clinical condition of the patient could not be assessed properly. We didn't speciate the isolates.

Conclusion

Over the last four years there has been a persistent rise in the methicillin resistance among CoNS isolated from blood culture specimens in our set up. These are usually resistant to many other commonly used antibiotics leaving limited treatment options.

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In vitro Efficacy of Azithromycin against *Salmonella typhi*

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Abstract

With the emergence of multidrug resistant and reduced susceptibility of *Salmonella typhi* isolates to fluoroquinolones and resultant treatment failure in enteric fever patients, there is an urgent need to establish alternative antibiotic treatment. Azithromycin has shown promise in treatment of typhoid fever. This study was aimed at detecting *in vitro* efficacy of azithromycin against multidrug resistant and nalidixic acid resistant *Salmonella typhi* in our set up.

Methodology

This study was conducted at the department of Microbiology, Army Medical College, Rawalpindi from December 2006 through August 2008. *Salmonella typhi* was isolated by routine microbiological methods from blood cultures. Susceptibility was determined by standard disc diffusion method. For minimum inhibitory concentration E-strips of azithromycin were used.

Results

A total of sixty two *Salmonella typhi* isolates were included. Isolates sensitive to first line antibiotics were 28(45.2%), multidrug-resistant but nalidixic acid sensitive were 6 (9.7%), multidrug-resistant and nalidixic acid resistant 2 (3.2%), and only nalidixic acid resistant 26 (41.9%). Minimum inhibitory concentration of Azithromycin for all isolates was in the sensitive range *i.e.* below 16µg/ml. The mean minimum inhibitory concentration of the isolates sensitive to the first line antibiotics was 2.5µg/ml whereas for the multidrug-resistant & nalidixic acid resistant isolates it was 3.3µg/ml.

Conclusion

Our study showed an increase in the minimum inhibitory concentration of the multidrug-resistant and nalidixic acid resistant *Salmonella typhi* isolates. Azithromycin can be beneficial in the treatment of enteric fever caused by multidrug-resistant and nalidixic acid resistant isolates of *Salmonella typhi*.

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Key Words

Azithromycin, Minimum Inhibitory Concentration, Multidrug Resistant *Salmonella typhi*.

Introduction

Enteric fever caused by *Salmonella typhi* remains a major cause of morbidity and mortality worldwide. It is endemic in the developing countries, especially in Southeast Asia and Africa. First line therapy comprising ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol no longer provides reliable coverage because of emergence of multidrug-resistant (MDR) isolates. Fluoroquinolones have proven to be effective for the treatment of typhoid fever caused by MDR strains, but *Salmonella* strains resistant to fluoroquinolones have already been reported. Further, due to reduced susceptibility of fluoroquinolones failures of clinical treatment of typhoid patients with ciprofloxacin and other fluoroquinolones are being reported.¹⁻⁶

There is an urgent need to establish alternative antibiotic treatment. Azithromycin has shown promise in treatment of typhoid fever, having once daily dosage and short duration of treatment.⁷⁻⁹ It is the first of a new class of broad spectrum antibiotics called azolides which are derivatives of the basic macrolide nucleus. It has MIC of 4-16 mg/L against isolates of *S. typhi*. Rapid movement of azithromycin from blood into tissue results in higher concentration in tissues; 50-100 times the concentration in plasma. After oral administration, serum concentration declines resulting in 68 h half-life. Prolonged concentration in cells is advantageous in treatment of *Salmonella* species in mice and may explain the good results obtained in typhoid fever patients.^{10, 11}

This study was aimed at detecting the *in vitro* efficacy of azithromycin against MDR and nalidixic acid resistant clinical isolates of *S. typhi* in our set up.

Material and Methods

This study was conducted at the department of Microbiology, Army Medical College, Rawalpindi from December 2006 through August 2008. Blood from patients suspected of typhoid

fever was cultured in Brain Heart Infusion (Difco) broth. *S. typhi* isolates were identified by API 20E (API system BioMerieux, France) and confirmed serologically by using standard antisera (Bio-rad). The isolates were stored at -20°C.

First line antibiotics tested were ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. MDR isolates were defined as resistance to these three first line drugs. Sensitivity was tested by using standard disc diffusion methods as recommended by Clinical Laboratory Standards Institute.¹² Ampicillin (10µg), co-trimoxazole (1.25/23.75µg), chloramphenicol (30µg), and nalidixic acid (NA) (30µg) discs were used.

For Minimum inhibitory concentration (MIC) determination of azithromycin, E-strips (AB Biodisk) were used. Turbidity used was 0.5 McFarland turbidity standard (10⁶ cfu/ml), and petri dishes incubated for 18-24 hours at 37°C aerobically. *Escherichia coli* ATCC 25922 was used as control strain. The MIC range was 4-16µg/ml against isolates of *S. typhi*.^{2,10,12} The results were interpreted according to manufacturer's instructions.

Results

The susceptibility pattern of all the isolates is shown in table 1. Isolates sensitive to first line antibiotics were 28 (45.2%); MDR and NA sensitive 6 (9.7%); MDR and NA resistant 2 (3.2%); and only NA resistant but first line sensitive 26 (41.9%). Total number of MDR in combination with NA resistant or alone were 8 (12.9%) and total NA resistant isolates in combination with MDR or alone were 28 (45.16%).

MIC of Azithromycin for all 62 (100%) isolates was in the sensitive range *i.e.* below 16µ g/ml (Table 2). The mean MIC of the isolates sensitive to the first line antibiotics was 2.5µ g/ml and that of MDR & NA resistant isolates 3.3µg/ml.

Discussion

Drug resistance is fast becoming a major problem in the management of infections caused by typhoidal salmonellae. MDR *S. typhi* have been endemic in most of South East Asia and the Indian subcontinent for many years. Data from many endemic areas is sparse and with the increasing problem of reduced sensitivity to the fluoroquinolones, empirical choice of antibiotics may be difficult.¹⁰ Our study indicated an increase

Table 1: Susceptibility pattern of isolates of *S. typhi* (n=62)

Sensitivity Type	n(%)
1 st Line Sensitive + NA Sensitive	28(45.2%)
MDR + NA Sensitive	6(9.7%)
MDR + NA Resistant	2(3.2%)
NA Resistant + 1 st line Sensitive	26(41.9%)

Table 2: MIC for Azithromycin against *S. typhi* isolates

<i>Salmonella typhi</i> (n=62)	Mean MICµg/ml (Range)
1 st Line Sensitive+NA Sensitive (n=28)	2.5 (1.5-4)
1 st Line Sensitive +NA Resistant (n=26)	2.0 (1.0-4.0)
MDR + NA Sensitive (n=6)	2.4 (1.5-6)
MDR + NA Resistant (n=2)	3.3 (1.0-8)

in nalidixic acid resistance in these isolates. This is in agreement with many local and international studies.^{1,14-17} Studies from various areas of Pakistan, India and Nepal show that isolates of *S. typhi* are on the rise and this trend is in accordance with our study, however, in one study, done locally, *S. paratyphi A* isolates were dominant.¹⁸⁻²¹

Evaluation of antibiotics which are cost effective, compliant, and easily available should be our first priority. Most importantly the choice of these antibiotics must be based on current trends of susceptibility patterns. Our results showed that azithromycin was effective against all isolates of *S. typhi* irrespective of their sensitivity to the first line antibiotics or MDR or nalidixic acid resistant. Clinical trials conducted through the last few years have shown that azithromycin can be successfully used in clinical settings in comparison to chloramphenicol, ceftriaxone and fluoroquinolones, providing comparable or better results as far as duration of treatment, reversal of symptoms and relapse was concerned. The conclusion is that azithromycin can be used successfully in MDR, and nalidixic acid resistant strains of *S. typhi*, causing uncomplicated infections. With once daily dosage for seven days, the fever clearance time was shorter, post treatment fecal carriage was low (about 2%) and the antibiotic was well tolerated with only minor side effects. No relapses have been reported.²²⁻²⁴

The *in vitro* activity of azithromycin against *S. typhi* in our study is similar to other studies.^{7,25} This is much higher than the levels achieved in serum by azithromycin and can be explained by the fact that the intracellular concentration is much higher.^{10,11} In addition azithromycin remains in tissues for 3-7 days post treatment due to its prolonged half-life.⁷ Compliance, cost, availability, efficacy and safety need to be considered when choosing antibiotics for treatment in developing countries with limited resources where disease is endemic due to poverty and azithromycin seems to fit the bill perfectly.

Apart from the fact that all isolates were sensitive to

azithromycin, a pertinent finding of our results was a slightly raised mean MIC of azithromycin against all resistant isolates. This means MIC of 3.3µg/ml though well within the sensitivity range but slightly raised as compared to the mean MIC of 2.5µg/ml of the sensitive isolates. Studies with larger sample size would give us a better perspective.

Conclusion

Azithromycin will prove beneficial in the treatment of enteric fever caused by sensitive, MDR and nalidixic acid resistant isolates of *S. typhi*. The lower cost, easy administration and short duration of treatment can decrease the burden of treatment and prove beneficial in treating enteric fever.

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Potential Predictors of Post-Caesarean Wound Morbidity and Characteristics of Microorganisms

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Abstract

Introduction

Worldwide rate of Caesarean section has been increasing beyond the recommended level of 15% by World Health Organization. The short and long-term morbidities of the rising Caesarean section rate on the childbearing population are conflicting as compared with international standards. Wound morbidity increases length of hospital stay and is associated with increased costs to the healthcare system.

Objective

To identify the potential predisposing risks factors for high Caesarean section rate, Caesarean section wound morbidity and characterize organisms causing wound infection and its relation between antenatal care.

Design

Descriptive prospective study.

Setting

Jinnah Medical College and Teaching Hospitals (JMCH), Department of Obstetrics & Gynecology, Karachi, Pakistan.

Method

All the women, delivering by Caesarean section (both emergency and elective) at JMCH, from 1 January 2011 to 15 June 2012 were included. Data was collected, on a specially designed questionnaire after taking informed consent from the patients who developed post-Caesarean complications during the study period. The isolated microorganisms were identified along with antimicrobial susceptibility.

Results

During the study period, there were 1012 deliveries with an overall Caesarean section rate of 38%. The rate of Caesarean section wound morbidity was 26%. In most of the patients, wound complication was disruption that was evident within first five days after delivery. Growth was yielded in 28% patients. Of 94 isolates, 41% were resistant to first generation cephalosporin and 15% were resistant to ampicillin,

metronidazole and gentamicin.

Conclusion

The frequency of Caesarean section is high in our setup and increasing each year. Approximately, one-quarter of abdominally delivered patients developed wound complication. A significant portion of pathogens causing post Caesarean abdominal wall wound infection were resistant to prophylactic treatment and some to empirical treatment.

Key words

Caesarean Section, Post-Caesarean Morbidity, Wound Complication.

Introduction

Worldwide rate of Caesarean section (CS) has been increasing beyond the recommended level of 15% by World Health Organization (WHO) as being optimal, for best outcome of babies and mothers.^{1,2} High CS rate have been reported not only in developed countries but also in developing countries.³ CS rate in USA is 29.1%, England 21.5%, Brazil 50.8%, China 37.7%, and India 32.6%^{8,4-7} Data available for Brazil show that the overall rate of CS for the country as whole is 30% of all deliveries, reaching as high as 50% of all deliveries in certain provinces.^{8,9} In this situation other factors including malnutrition and poor social conditions are likely to exacerbate the already higher risk of infectious morbidity and mortality associated with CS. Another serious concern is the fact that a considerable number of CS are unnecessary and planned in advance, with the additional potential risk of iatrogenic prematurity. Infants born through CS are at increased risk for developing respiratory problems and sepsis as compared with those by normal delivery.

Infection at the surgical site after CS is still a common complication. The importance of preventing surgical site infections is well recognized due to increased morbidity and length of hospital stay, and associated increased costs to the healthcare system especially with increased resource utilization associated with prolonged antibiotic treatment, need for re-operation, and even re-admission. Antibiotic prophylaxis is recommended for all such operations.¹⁰ Antibiotic should be administered preoperatively, ideally within 30 minutes of anesthesia induction. Single-dose antibiotic prophylaxis is recommended for CS surgery following clamping of the umbilical cord.¹¹ Deciding which antibiotic is most suitable as

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a prophylactic for CS is important; ampicillin or first generation cephalosporin are the most appropriate drugs.¹²

As maternal death has become rare in industrialized countries, it is increasingly important to study severe maternal morbidity.^{13,14} Planned Caesarean deliveries are associated with significantly increased risks of specific severe postpartum complications (e.g., hemorrhage requiring hysterectomy, cardiac arrest, venous thromboembolism, major infection) relative to planned vaginal deliveries.¹⁵ Such severe morbidity requires particular clinical attention.¹⁶

This study was conducted to:

1. Determine the rate of CS along with wound morbidity.
2. Identify the potential predisposing risks factors for CS wound morbidity and characterize organisms causing wound infection.
3. Identify relation between antenatal care and the CS wound morbidity.

Materials and Method

This descriptive study was performed at Jinnah Medical College & Teaching Hospital from 1 January 2011 to 15 June 2012. After taking informed consent, data was collected on a specially designed questionnaire. Patients' medical records were reviewed for positive wound cultures and isolated microorganisms.

All women of any age and parity who underwent CS in our hospital were included. Cases with other associated morbidities were excluded.

Gestational age was determined by the last menstrual period or ultrasound dating, according to the recommendations by American College of Obstetrics and Gynecology.¹⁷

Wound complication can be defined as either a wound disruption or wound cellulitis. A wound disruption means partial or complete opening of the deep subcutaneous space. Superficial skin separation was not taken as wound disruption, and these cases were not included in wound complication. Underlying causes for wound disruptions included seroma, hematoma, abscess, and fascial dehiscence. For the purposes of this study, wound cellulitis was defined as a physician diagnosis of erythema and warmth spreading beyond the immediate area surrounding the incision and requiring treatment with antibiotics. Wound infection was defined according to the CDC (Centers for Disease Control) superficial and deep incision criteria.¹⁸

Booked patients had at least two antenatal clinic visits, and unbooked patients were seen for the first time in the emergency room in labor or at term. Data was collected, on a specially designed, questionnaire. The questionnaire form designed for this study included information about some variables that influence complication development like demographical features, anaemia, booking status, type of surgery, type and timing of

complications, and treatment required. Statistical analyses were carried out using the SPSS version 17.0.

Results

During the study period, there were 1012 deliveries with an overall CS rate of 38% (n=385). Out of these, 100 (26%) patients developed CS wound complication. The study showed that 68% of study population was between 20-34 years of age and about 65% lived in rural area. 61% were multipara with parity 1-4 (Table 1).

Majority of the patients with wound complication were unbooked (71%), anaemic (74%), had emergency surgery (76%), and postpartum hemorrhage (38%).

Among wound complication 85 % of were disruptions. 36% required readmission to the hospital for treatment, and 14% required re-operation because of wound complication (Table 2). All cases of re-operation required simple wound debridement.

Twenty-seven percent patients yielded isolates from wound culture. Of 94 isolated microorganisms, 41% were resistant to first generation cephalosporin (prophylactic treatment) and 15% were resistant to ampicillin, metronidazole and gentamicin (empirical treatment) as shown in table 3. Most of the complications were diagnosed within a week post-surgery and 22% within a month after surgery (Table 4).

Discussion

The key to successful management of postpartum complications is the identification of patients at risks and the adaptation of precautions to avoid morbidity and mortality, however in many cases complications occur unpredictably and successful management depends on early recognition and adequate timely treatment.

Table 1: Demographic characteristics

Characteristics	%age n=100
Age	
< 20 years	21 %
20-34 years	68 %
≥ 35 years	11 %
Region	
Rural	65 %
Urban	35 %
Parity	
Primi	18 %
1-4	61 %
≥5	21 %

Early and long-term complications are increased in women delivered by CS when compared with the outcomes after vaginal deliveries with risks of surgery and anesthesia.¹⁹ CS rate in this study was higher as compared to industrialized countries; United States 29.1% and England 21.5%. Main reason of high CS rate in this study was the fact that patients living near by our hospital

Table 2: Type of wound complication and management (n=100)

Characteristics	%age
Wound disruption	85 %
Wound cellulitis	12%
Management	
Out patient	64%
In-patient	36%
Conservative management	82%
Re-operation	15%

Table 4: Wound complications according to time of occurrence

Time of reporting	%age n=100
On first out patient	5%
Within a week	69%
Within a month	22%
After one month	4%

in Korangi (industrial area of Karachi) were poor with large family size. They couldn't afford antenatal care and consider birth as a natural process. Wives were brought to the hospital when they are seriously ill and insisted on vaginal delivery. Only those patients were referred to this tertiary care hospital who had one or more risk factors and who already had a trial of labour somewhere else. Literature review revealed that majority of unbooked patients landed up into emergency CS as in our study, and emergency procedures had greater incidence of

Table 3: Microorganism isolated and sensitivity pattern

Microorganism	Total	Prophylaxis		Empiric treatment		Resistant to empiric treatment
		Resistant to prophylactic treatment	Sensitive to prophylactic treatment	Ampicillin sensitive	Gentamicin sensitive	
<i>Escherichia coli</i>	24	6	18	8	19	8
<i>Enterococcus faecalis</i>	14	14	NR	14	NR	-
<i>Staph aureus (MSSA)</i>	12	-	NR	NR	12	-
<i>Staph aureus (MRSA)</i>	2	2	NR	NR	1	1
<i>Pseudomonas aeruginosa</i>	7	7	NR	NR	06	1
<i>Streptococcus viridans</i>	5	-	NR	NR	NR	-
<i>Klebsiella pneumoniae</i>	5	1	4	-	5	-
<i>Morganella morganii</i>	5	5	-	-	4	1
<i>Citrobacter koseri</i>	4	2	2	-	4	-
<i>Yeast</i>	3	NR	NR	NR	NR	3
<i>Proteus mirabilis</i>	2	-	2	-	1	1
<i>Enterobacter aerogenes</i>	2	1	1	NR	2	-
<i>Pseudomonas spp</i>	1	1	-	-	1	-
<i>Klebsiella spp</i>	1	-	1	-	1	-
<i>Enterobacter</i>	1	1	-	NR	1	-
Other Streptococci	6	-	NR	NR	NR	-

NR = not relevant

infection.²⁰

This study demonstrated that nearly one-quarter women had significant wound complication after CS delivery. Our results indicated that the risk of complication increases with anaemia, large family size, unbooked status, unawareness, poverty, emergency surgery, and postpartum hemorrhage. Some of these factors could have been modified with earlier intervention.

Anaemia plays an important role in both wound healing and host resistance to microbial contamination. Hyperoxia leads to increased collagen synthesis; while hypoxia has the opposite effect.²¹ A similar relationship has been observed in wound tensile strength, hyperoxemia promotes epithelialization and wound angiogenesis.^{22,23}

Large family size because of repeated pregnancies with no or short inter pregnancy interval leads to anaemia, poor maternal health, delayed wound healing, and wound complications.

Unbooked status had no counselling to improve maternal general health, build haemoglobin, and do adequate preparation for safe delivery, timely referral to tertiary care centre and adequate birth spacing. All these features and late referral lead to emergency surgery, more chance of post partum haemorrhage and wound complications.

Most Cesarean deliveries are clean-contaminated operations. However, the etiology of Cesarean wound complication is likely multifactorial and not fully described by the list of variables included in our study.²⁴ Other potential predictors of CS wound complication include methicillin resistant *Staphylococcus aureus* carrier status and the use of chlorhexidine for preoperative skin cleaning.²⁵

Our data demonstrated that the accurate capture of Cesarean wound complications requires reliable follow-up after hospital discharge because most of the patients were usually discharged, on request, on third post operative day and 64% wound complications occurred within a week after surgery. Previous publications relying on hospital discharge data or post-hospital discharge questionnaires likely underestimated the incidence of wound complication. Follow-up for our study involved documentation of physical examination, diagnosis, and treatment by a physician, thus ensuring the accuracy of our results. Our findings are comparative with other studies showing that majority of CS wound complications were diagnosed after hospital discharge.^{26,27}

Strengths of our study included a conservative definition of wound complication, and a relatively large sample size. Therefore, our results likely reflected the true burden of CS wounds. Weaknesses of our study included: retrospective study design, post discharge follow-up on limited number of patients, and lack of information on subcutaneous depth.

Conclusion

This study demonstrated that nearly one-quarter women had noteworthy CS wound morbidity. Our results indicated that the risk of complication increases with anaemia, large family size, unbooked status, emergency surgery, and postpartum hemorrhage. Significant portion of the pathogens causing wound infection after abdominal delivery were resistant to the conventional prophylactic treatment, and some were resistant to empirical treatment.

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Molecular Diagnostic Assays Detection of Crimean-Congo Haemorrhagic Fever Virus

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Abstract

Viral hemorrhagic fever caused by Congo-Crimean Haemorrhagic Fever Virus (CCHFV) and dengue virus is endemic in Pakistan. Both CCHF and dengue can occur simultaneously and this poses a problem in patient placement and management in the hospital. Rapid molecular based diagnosis of CCHF based on a reverse-transcription based polymerase chain (RT-PCR) assay is described. The study describes both a nested- conventional PCR based assay for CCHF detection which has a turn-around time of 24- 48 h and also a real-time PCR based assay which has a turn-around time of 2 - 4 h. The utility of these tests in diagnosing and managing CCHF infections is reviewed. Rapid identification of positive cases together with screening out of suspected CCHF cases which are CCHF negative have implications for management in the hospital in terms of risk of transmission, nursing, infection control and also costs of treatment.

Key Words

Congo-Crimean Haemorrhagic Fever Virus, Nested Conventional Polymerase Chain Reaction, Reverse Transcription - Polymerase Chain Reaction.

Introduction

Crimean-Congo Hemorrhagic Fever virus, a member of the Nairovirus genus of the family Bunyaviridae, is an RNA virus. According to the most recent report from the International Committee on the Taxonomy of Viruses, there are seven recognized species in the genus Nairovirus containing 34 viral strains. CCHF is endemic in Middle-East and Pakistan.¹ Initial symptoms of CCHF are fever, diarrhoea, vomiting, severe headache, dizziness, throat pain, nausea. In severe condition, symptoms are bleeding under skin, petechiae, ecchymosis, conjunctivitis, bleeding in internal organs and orifices resulting in a case fatality rate of approximately 50%.²

Both CCHF and dengue can occur simultaneously and this poses a problem in patient placement and management in the hospital. CCHFV spreads through contact with blood and mortality after nosocomial acquisition is unacceptably high. Moreover, healthcare workers exposed to CCHFV require

prompt attention and post-exposure prophylaxis with Ribavirin, along with its attendant cost and adverse effects.

Mode of Transmission

CCHF infection is spread via the bite of *Ixodid* ticks (genus *Hyalomma*) mostly through livestock. Human can become infected through contact with infected livestock or direct contact with infected blood or tissue (Fig 1). The viral incubation period is about 2-9 days.³

Virion Structure

Virions are spherical, approximately 100 nm in diameter, and have a host cell-derived lipid bilayered envelope approximately 5-7 nm thick, through which protrude glycoprotein spikes 8-10 nm in length. CCHF virus possesses three negative-sense RNA genome segments: the large (L), medium (M), and small (S) segments which are complexed with nucleocapsid protein to form ribonucleocapsid structures. The nucleocapsids and RNA-dependent RNA polymerase are packaged within a lipid envelope that contains the viral glycoproteins, G1 and G2 (also referred to as Gn and Gc, respectively (Fig. 2)).⁴ The S segment encodes a nucleoprotein (NP), the M segment encodes a glycoprotein precursor, which is cleaved into mature glycoproteins, G1 and G2, and the L segment encodes RNA polymerase.⁵

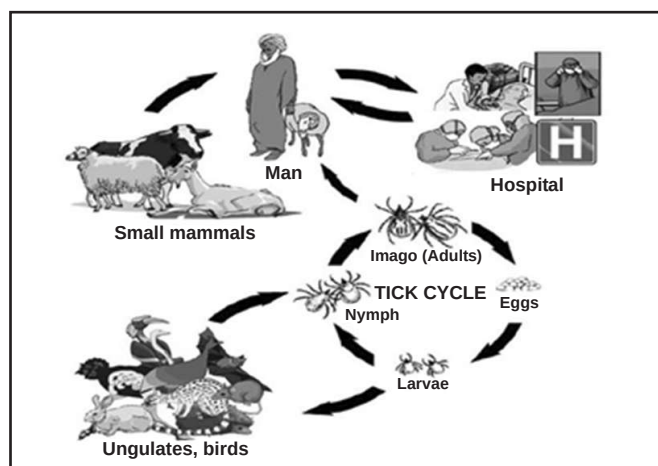


Fig 1: Example of CCHF virus circulation: transmission by the *Hyalomma marginatum rufipes* ticks. Adapted from Nasbeth P.²

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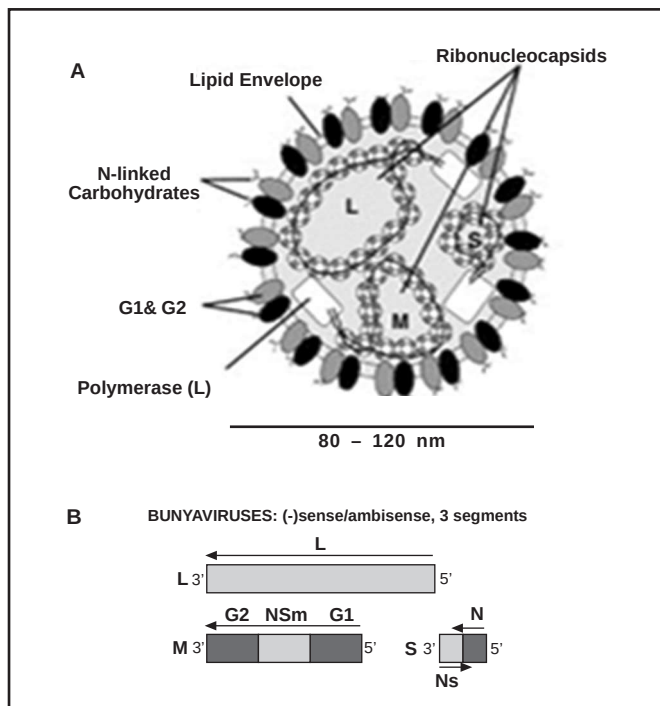


Fig 2 : Congo-Crimean Hemorrhagic Fever Virus (CCHFV) structure

- A.** Viral nuclear envelope with lipid and carbohydrate moieties. Internal structure of virus comprising essential proteins such as polymerases and ribonuclear envelopes surrounding the L (large), M (medium) and S (small) segment RNAs are illustrated in a figure adapted from Whitehouse et al.
- B.** Illustration of the RNA genome segments of CCHFV comprising single stranded anti-sense RNA of L, M and S segments.

Diagnostic Methodology

Serology

CCHF can also be diagnosed by serological methods based on an enzyme-linked immunosorbent assay (ELISA) to proteins from the CCHF virus. Thus, assays developed include detecting CCHFV induced immunoglobulin G (IgG) antibodies to the recombinant nucleoprotein (rNP) whereby immunoglobulin M (IgM)-capture ELISA detects CCHFV rNP as an antigen.⁶ Additionally, a novel monoclonal antibody based ELISA which detected the CCHFV rNP was found to be more sensitive to detect CCHF infection in sera of patients with acute disease.⁷ However, serological assays are limited by the time period which is required for the host to generate an antibody response, which is approximately 5 days or more. Hence, while serological assays are useful for screening for CCHF in the population and for identification of sub-clinical infections these may not be useful in the acute phase of disease.⁸ In active disease viral RNA would be present while there may be absence of antibody responses and therefore, molecular based methods of detection

may be more suitable.

Molecular Methods

Nested PCR

In the first step RNA isolated from clinical specimens was reverse transcribed by a reverse transcriptase (RT) to prepare cDNA. RNA was extracted using the Nucleo-Spin RNA extraction kit and eluted into 50 µL of total volume. Ten µL of RNA was reverse-transcribed using Fermentas RT-PCR reagents. RT was performed using primers targeting the S segment of the genome as described previously.⁹ The product of the first round of PCR was used as template for the second 'Nested' PCR. The subsequent PCR product was detected after agarose gelelectrophoresis (Fig. 3A).

Real Time PCR

Realtime PCR can be combined in a one-step reaction with to quantify low abundance messenger RNA. Here, RT-PCR was performed directly on viral RNA using sequence specific primers and a fluorescent probe for CCHF on Light Cycler II system, Roche, USA as described previously (Fig. 3B).¹⁰ In the One-step realtime PCR amplification method RNA eluted from clinical samples was directly used in the RT-PCR reaction. Briefly, 10 µL of RNA was transcribed using Super Script III One step RT-PCR reagents (Invitrogen, USA). Target CCHFV cDNA was detected using a fluorogenic probe based method whereby fluorescence measurement of the PCR was detected at the end of each cycle.

Comparison of Molecular Assays

A comparison of the nested PCR and realtime PCR based RT-PCR assays for CCHFV detection indicate that real-time PCR assay allows a faster reporting time, within 3 h, while the nested PCR requires 10 - 12 h of laboratory testing as it has three separate steps for reverse transcription, and the two PCR assays (Fig. 3C). In addition, the real-time PCR assay can detect as few as 10 CCHFV RNA genomes and is therefore more sensitive than the nested PCR assay.¹⁰ Importantly, the real-time PCR assay is a closed tube system and once the protocol is set up it does not require further handling, while the nested PCR assay requires subsequent manipulation of RT assay and set up of secondary PCR. This results in a reduced risk of contamination in the realtime PCR assay as compared to that of the nested PCR assay.

Conclusions

Viral hemorrhagic fever caused by CCHFV and dengue virus (and possibly Chikungunya virus) is endemic in Pakistan.^{11,12} Rapid confirmation of CCHF allows institution of appropriate measures for prevention of cross transmission and patient management and hence constitutes the single most important measure for in-hospital management of CCHF. Provision of facilities for CCHF detection by Real-time PCR is mandatory in endemic areas.

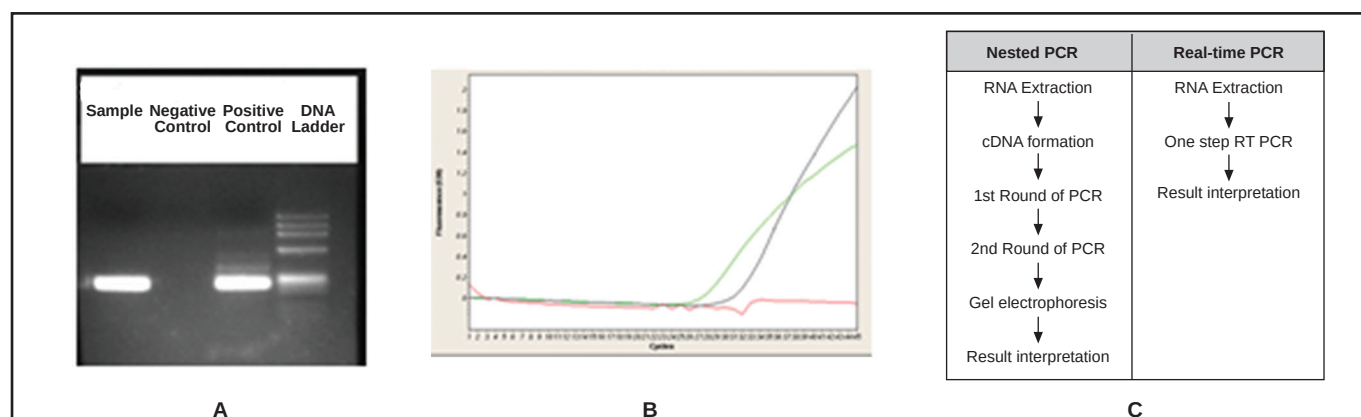


Fig 3 : Comparison of Nested PCR and Real-time PCR based detection for CCHF virus.

A. Interpretation of nested-PCR based detection of CCHFV RNA on an agarose gel is illustrated.

B. The figure shows detection of CCHF virus using a fluorogenic probe with a 5'FAM label and 3' BHQ label to the S segment genome as determined by the LightCycler II software version 3.0. The figure denotes a positive (CCHF virus RNA plasmid, black line), negative (non-template control, red line) and test sample (green line).

C. The different steps in each protocol are identified by the flow diagrams in each case.

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When not to Use Antibiotics? - A simple guide for prevention of misuse of antibiotics

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Abstract

Antibiotics are lifesaving but have been misused so much that they are losing their utility due to major resistance emerging worldwide. Physicians, general practitioners, pediatricians and other disciplines are all equally responsible for this inappropriate misuse in mostly viral infections due to many reasons. However, simple key points of signs and symptoms in history, physical examination, vaccination status, exposure, epidemiology like age or season, underlying immunosuppression or previous treatment history will allow judicious use of antibiotics for common infections. The prudent physician must balance the risks and benefits of treating with antibiotics versus not treating a particular illness episode in an individual child especially if viral in origin. Emphasis must be on understanding that common viral infections do not require use of antibiotics. Here we will review some of the factors that will help in curtailing the misuse of antibiotics in common practice and reduce its associated adverse outcome.

Key Words

Antibiotic Misuse, Antibiotic Resistance, Viral Infection, Upper Respiratory Infection

Typical Case Scenario

A 10-month-old girl is brought by her mother with complaints of repeated upper respiratory tract infections (URTIs). She says the child had already 6-7 episodes of high fever and coughs and had multiple antibiotics courses (including, cefixime thrice, cefaclor twice and clarithromycin twice). These episodes are almost same with mostly fever initially followed by cough, rhinorrhea that lasts 5-7 days and the child has never been hospitalized. The child on examination is febrile but playful with runny nose and pharyngitis. Her growth and development is normal. Immunizations are complete to date. The mother is upset and wants to know what is wrong with her child, what can be done to prevent and what tests or immune workup does she need.

This is a typical case of antibiotic misuse as almost all of these are viral URTIs. These are self-limiting illnesses that require symptomatic treatment only and reassurance.

Introduction

We know that antibiotics save millions of lives including

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children. Some facts and figures of antibiotic overuse are staggering. Approximately two-third (68%) of antibiotics are prescribed for URTIs.^{1,2} However >80% of such prescriptions have been found to be unnecessary and inappropriate with adverse outcome including the menace of antibiotic resistance.

As astute physicians we must be judicious in our antibiotics prescribing practices whether we are working in clinics, hospitals or communities. There are many flimsy reasons for giving antibiotics. Is it then possible to limit our use of antibiotics to only those that are clinically indicated? We often ask, is it viral or bacterial? In our busy practices it may sound difficult as we are pressed for time, fear litigation, dread missing a potential fatal infection, may make poor judgment in diagnosing likely etiology or are under pressure from patients or parents.

However, by analyzing simple key points of signs and symptoms in history, physical examination, vaccination status, exposure, epidemiology like age or season, underlying immunosuppression or previous treatment history will allow to judiciously use antibiotics for common infections. A careful analyses of all these factors and confidently communicating the care plan to a worried parent, often over the cries of a fussy toddler during a 10-minute office visit, requires patience and thoughtfulness. There are many myths and misconceptions about viral infections and antibiotics. Common myths as below must be dispelled by intervention and measures:

- **Myth:** Cold and flu symptoms will feel better or get better faster on antibiotics.
Fact: Antibiotics cannot ease the symptoms of viral illnesses; these infections resolve on their own. Only symptomatic relief is helpful.
- **Myth:** Illnesses with the same symptoms require antibiotics.
Fact: Illnesses with similar symptoms can be caused by infections (including viral) and non-infectious.
- **Myth:** If I take an antibiotic, I won't spread my illness to others.
Fact: Viral illnesses (colds and flu) usually are spread from person to person before the onset of symptoms. Antibiotics cannot stop this spread.

The following section will focus on URTIs and other viral infections and briefly describe how some of the key concepts, rationale and careful thinking will help in reducing our antibiotics prescriptions in children. Remember that rarely severe infections and complications may arise in these viral infections that require specific therapy, which we will not discuss in this section.

History

Key points in history that help in diagnosing viral infection should be sought especially duration and initial symptoms.³⁻⁵

A typical patient will come in with a short history of high-grade fever, cough, itchy or red eyes and headache. Sometimes associated symptoms include vomiting, diarrhea and anorexia. Specifically sequence of events is important.

A mild to high-grade fever for first few days followed by cough and rhinorrhea is the normal course for a viral infection. Some cough and rhinorrhea may last longer and usually resolves within 7-14 days.⁶⁻⁸ It also means that early high spiking fever is common in viral infection.

Most sore throats or pharyngitis are due to viruses. Sometimes *Streptococcus pyogenes* (Group A Strep) may be suspected in typical school age (4-15 yrs.) but have no associated cough or rhinorrhea. Uncommonly, sinusitis may occur in the setting of URTI in older children and should be suspected if low-grade fever, headache or hacking persistent cough occurs beyond 10 days. Similarly watery diarrhea even after a febrile period signifies a self-limiting viral etiology. Severe URTI symptoms with myalgia and fatigue point to influenza or parainfluenza virus as possible etiology.

Duration of illness must be one of the first considerations. The natural history of *rhinovirus* infection in children and young adults shows only mild or no fever initially but rhinorrhea lasts sometimes beyond 14 days in 25% patients.^{3,4} Most patients will have recovered in 7-10 days.

Prolong fever with or without other symptoms points to a bacterial or other non-infective cause. Empiric antibiotics should be avoided in such cases pending full evaluation. Keep in mind that repeated "interrogations" about an illness and examinations might bring forth some hidden aspects of history.

Physical Examination

Single or multiple examinations may be necessary to detect findings that will aid to a specific diagnosis of a specific infection. For example a lack of toxicity despite high-grade fever in a child who specially may be playful points to a benign viral infection. In children, it is mandatory that a good examination of the ears, eyes, nose and throat be always done. It will mostly reveal the viral nature with evidence of conjunctivitis, pharyngitis, coryza or simple erythema of the tympanic membranes bilaterally.

A common viral sign that should be sought is presence of a rash during the course of illness or at presentation. A maculopapular erythematous rash that mostly follows a febrile period points to roseola (due to *human herpes simplex virus* 6). Similarly, the typical exanthems such as that associated with varicella may be seen. Diffuse small lymphadenopathy or transient mild hepatosplenomegaly may also accompany such

an illness. Rarely arthritis or meningeal signs may also be present.

Briefly a previously healthy child with fever with or without viral symptomatology and non-toxic on examination most likely has a self-limiting viral illness and does not require an antibiotic.

Syndromic Diseases

Most childhood illnesses are viral and have a particular presentation that gives a clue to the etiological organism.⁵ It should take into account the age, season, sequence of events and findings on examination and sometimes laboratory clues that points to the infecting organism. The age of the patient may also be helpful in pointing to a specific viral etiology as below:

Etiology: Most common causes by age

1. Age under one year
 - a. Respiratory Syncytial Virus (RSV) (winter to spring)
 - b. Parainfluenza (fall)
 - c. Coronavirus (winter to spring)
2. Age 1 to 10 years
 - a. Parainfluenza (fall)
 - b. Enterovirus (fall)
 - c. RSV (winter to spring)
 - d. Rhinovirus (fall)
3. Age over 10 years
 - a. Influenza virus (winter to spring)
 - b. RSV (winter to spring)
 - c. Adenovirus

The following are some of the viruses that cause characteristic syndromes in children (see appropriate sections for details):⁵

• Bronchiolitis (<2 years old)	RSV
• Measles	Rubeola virus
• Croup	Parainfluenza viruses
• Herpangina (hand-foot-mouth)	Coxsackie A viruses
• Pharyngoconjunctival fever	Adenoviruses
• Infectious mononucleosis	CMV

Burden of Disease

Most URTIs and other diseases in both adults and children are viral and are self-limiting. Multiple large epidemiologic and community based studies from early 20th century have documented common viruses that are responsible for the major burden in all age groups.^{3,4,6-12} Just the simple fact that these infections are so common that a particular child with the typical presentation most likely may have a benign viral illness for which no particular therapy is indicated including using antibiotics. These viral illnesses include *Varicella* (chicken pox), *Rubella* (German measles), the common cold, bronchiolitis, pharyngitis, hepatitis A, mumps, infectious mononucleosis (EBV), *Rubeola* (measles). Obviously, in these antibiotics will

not work or change the course of the illness. Respiratory viral illnesses cause burden in all age groups. The burden of uncomplicated URIs is approximately as below:¹³

- 25-30% *Rhinoviruses*
- 25-35% *RSV, Parainfluenza* and *Influenza* viruses, the newly described *Human Metapneumovirus* and *Adenoviruses*
- 10% *Corona* viruses
- 20-25% Other / unidentified viruses

While the community acquired pneumonia incidence is estimated to be 30-40 per 1000 person-years that of the common cold is 2-4 per person per year and acute sinusitis complicates only about 0.5% of colds (10-20 /1000 person-years).¹⁴

Season

Most viruses follow a seasonal pattern.^{10,11} These viral infections occur predominantly during certain months throughout the year and help in narrowing possible etiology as below:

Virus	Month
• <i>Rhinovirus</i> (1 st peak)	March and April
• <i>Parainfluenza</i>	October and November
• <i>RSV, Influenza</i> viruses, and <i>Corona</i> viruses	Winter months
• <i>Adenovirus</i>	Continuously in cold months
• <i>Rhinovirus</i> (2 nd peak)	September

RSV and *Influenza* infection are common during winter months. The most common cause of viral lower respiratory tract infection (LRIs) in children is *RSV*. Unlike *Parainfluenza virus*, *RSV* tends to be highly seasonal. The *RSV* burden is also tremendous during peak winter months with overall 33% prevalence, total hospitalization 38%, and accounting for 77% infants < 6 months of age.¹⁵ Similarly population-based surveillance for *Influenza* showed that during peak winter season the hospitalization rate was 0.9 per 1000 children, clinic visits 50-95 visits/ 1000 children, and emergency visits 6-27/1000 children.¹⁶ In some tropical countries, *Influenza* viruses circulate throughout the year with one or two peaks during rainy seasons.

During summer months enteroviral disease in infants and children particularly presents with febrile illnesses, febrile seizures, URTI, exanthems, GI manifestations and other clinical manifestations.¹⁷ These are self-limiting and only need symptomatic treatment but may have significant economic impact.

Epidemics

Many viral infections in children tend to cluster and have epidemics.¹ Children will present in large numbers with almost same clinical manifestations. *RSV* occurs as winter and fall epidemics in infants. A number of infants will be seen with

acute respiratory distress, wheezing and minimal fever. Clinical and epidemiological risks will point to *RSV* etiology.

The *Influenza virus* circulates worldwide and can affect any age group. It causes annual epidemics that peak during winter in temperate regions. It has the potential to cause a serious public health problem with severe illnesses and deaths for higher risk populations including children. Clinically influenza must be differentiated from other mild viral illnesses as it may have complications. Mostly influenza epidemics occur yearly during autumn and winter in temperate regions. Illnesses result in hospitalizations and deaths mainly among high-risk groups (the very young, elderly or chronically ill). Worldwide, these annual *Influenza*-associated epidemics result in an estimated 28,000-111,500 deaths in children younger than 5 years, with 99% of these deaths occurring in developing countries.¹⁸ Most deaths associated with influenza in industrialized countries occur among people age 65 or older.

Currently (December 2011) the explosive dengue fever outbreak in Punjab, Pakistan and other provinces crossed >35,000 cases with >350 deaths. Health care providers are advised to treat patients symptomatically and to observe standard infection control precautions. The panic among the public has been created due to lack of awareness about dengue. The numerous myths and misconceptions about dengue also need to be dispelled by active health education.

In classical "Dengue Fever" the major symptoms are high fever, severe headache, severe pain behind the eyes, joint pain, muscle and bone pain, rash, and mild bleeding (e.g., nose or gums bleed, easy bruising). It lasts 6-10 days with complete recovery. In case of febrile illness and other cases reported from the community one must include dengue fever as possibility. One must rule out other causes as well such as malaria and typhoid. There is no specific treatment for dengue infection. Patients should be advised to use analgesics (pain relievers) with paracetamol, rest, drink plenty of fluids, and consult a physician if they feel very sick. Remember there is no role of antibiotics or antivirals.

Vaccination Status

The efficacy of all vaccines against many illnesses has been established including bacterial (*Hib, Pneumococcus*) and viral (*Measles, Varicella*, hepatitis and *Influenza*). Overall the prevalence of occult bacteremia in the post-conjugate vaccine era has decreased. It is thus prudent that a less aggressive approach to the management of completely immunized, well-appearing, febrile children who do not have a focal source of infection appears reasonable.¹⁹ An unimmunized child is always at more risk to acquire bacterial or viral infections. Lack of a specific vaccination may point to a likely etiology.

Laboratory Evidence

Most fevers are viral and do not require any laboratory testing.

If laboratory evaluation is done then clues may give supporting evidence for/against viral or bacterial nature of an illness. For example, viral infections have normal WBC count. Mild to moderate leucopenia may also occur in viral infections with lymphocyte predominance with absence of toxic granulations. Atypical lymphocytes may be seen in infectious mononucleosis. A normal hemoglobin level mostly rules out malaria while high hemoglobin may point to dengue infection. Normal stool analysis in a child with acute watery diarrhea is mostly of viral origin.

New diagnostic tests have the potential to detect a wider range of established and newly discovered viruses with greater sensitivity. Better diagnostic tests that establish the cause of URTIs and LRTIs in children have the potential to reduce overall antibiotic use and improve the targeted use of antibiotics. New highly sensitive and specific molecular assays for the detection of respiratory viruses have moved into the mainstream of clinical testing that have potential for cost savings, preventing hospitalization, decreasing length of hospitalization, decreasing unnecessary testing and procedures, directing specific therapy and reducing unnecessary antibiotic use.²⁰ In addition, rapid identification of viral infections can help control epidemic and nosocomial transmission.

A “Patient Waiting”

As physicians, prescribing an antibiotic for an acute illness in a child may be challenging. We all come across multiple pressures such as a diagnostic uncertainty, fear of the unknown, lack of knowledge about course of benign viral illnesses, lack of patient follow-up and of course possible litigation. We are “forced” in giving an antibiotic “cover” expecting that the patient will be satisfied with an antibiotic or to protect for “super-infection.” All these are myths and have lead to a multitude of other problems such as increased costs, antibiotic resistance and side effects.

Most practitioners recognize that most URTI illnesses are viral but some believe that lower respiratory bacterial super-infections might be averted by prophylactic use of antibiotics. Multiple trials and a meta-analysis have concluded that antibiotics do not prevent or decrease the severity of such bacterial complications.²¹ Patients would rather like to have assurance that antibiotics are not indicated and discussion about natural course of viral illness will suffice to satisfy the patients or their parents. It has been noted that patient satisfaction was predicted by time spent by doctor explaining illness and patient understanding of treatment choice (supportive care in common viral infections) and not predicted by receipt of antibiotics.^{22,23} There was a high rate of antimicrobials prescription (62% versus 7%) if physicians thought a parent wanted an antibiotic or not.²³

More than 75% of the children presenting with an URTI did not have a presumed or proven bacterial infection, require antibiotic or an increase in bacterial infections in one study.²⁴ A Cochrane review for common cold and acute purulent rhinitis

failed to justify antibiotics.²⁵ More recent studies have shown and recommended that antibiotics are less useful even for otitis media, sinusitis and bronchitis.²⁶⁻²⁸

Mostly strategies used to combat the increasing use of antibiotics have proven very effective such as no antibiotic in common viral infections, deferring an antibiotic prescription or a close follow up.²⁹ Moreover, a Cochrane review of 7 trials has shown 80% spontaneously resolution in otitis media and that patients are better spontaneously by day 7 regardless of treatment and the absolute benefits of antibiotics are somewhat difficult to ascertain.³⁰

Prevention

Most viral and other self-limiting viral illness in children can be prevented by simple infection control measures such as hand washing and avoiding contact with an infected patient. Also the widespread use of vaccines against measles, diphtheria, pertussis, *Hemophilus influenzae*, pneumococcus, and influenza has the potential to substantially reduce the incidence of respiratory infections in children in developing countries. Safe and effective vaccines have been available and used for more than 60 years. WHO case-management approach and the wider use of available vaccines will reduce respiratory infections mortality among young children by half to two thirds.³¹ Vaccination is especially important for people at higher risk of serious complications. All these measures must be accompanied by patient education (educational materials, discussion, using prescription for non-antibiotic therapies) and physician education (guidelines for management of common infections, continuous medical education and other educational activities, feedback on prescribing rates, rising rates of antibiotic resistance etc.).^{32,33}

Conclusion

The prudent physician must balance the risks and benefits of treating with antibiotics versus not treating a particular illness episode in an individual child. He/she must take into consideration the historical and physical details, age, season, prior health status, treatment history, daycare attendance, cost of antimicrobial agents or exposure. Emphasis must be on the understanding that most common viral infections do not require use of antibiotics. We hope the above brief points will help in curtailing the misuse of antibiotics in common practice and reduce its associated adverse outcome.

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Actinomycosis, a clinical masquerader: case report

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Introduction

Actinomycosis is an indolent, slowly progressive, granulomatous infection caused by bacteria of the genus actinomycetes that usually spreads into adjacent soft tissues without regard for tissue planes or lymphatic drainage.¹⁻³ Actinomycetes, first described in the 19th century, are ubiquitous in soil and as commensals of the mouth, gut, bronchial tree and genital tract.¹ The species name which means “Ray Fungus” in Greek is a misnomer as actinomycetes are true bacteria. It is derived from their hyphenate like, filamentous arrangement resembling fungi.⁴ Actinomycosis can affect any organ and there are different clinical presentations but their incidence has remarkably declined in recent years, especially in the developed countries. However, due to poor dental hygiene, limited access to antibiotics and poor socioeconomic conditions it is still a problem in the developing countries. The oral cervicofacial form accounts for 50% of the cases and pulmonary involvement approximately 15% of cases.⁵ Actinomycetes are usually anaerobic but a morphologically similar group, the aerobic actinomycetes, have been recognized as significant human pathogens in both immune-competent and immune compromised hosts causing similar spectrum of disease.⁶ We report a case of a young girl diagnosed with thoracic and soft tissue actinomycosis where the causative organism was aerobic actinomycetes.

Key Words:

Aerobic Actinomycetes, Filamentous Rods, Sinus Tracts, Sulfur Granules.

Case Report

A 20-year-old female presented to the Infectious Disease Clinic at the Indus Hospital on 24th February 2012 with a history of high grade, intermittent, non-localizing fever with chills for four months. It had been treated elsewhere with a course of amoxicillin-clavulanic acid prescribed for fortnight. The fever had subsided but she developed a lump on the upper abdomen which was painless and gradually increasing in size. Two weeks later another lump appeared below the first one and then a third appeared below the right clavicle which was oozing purulent material. She also had dry persistent cough, undocumented weight loss and anorexia for four months. There was no past history of tuberculosis or known contact with the disease.

On examination there was a large cystic swelling in the right

upper chest which was fluctuant but not inflamed or tender (fig 1). There was no cervical or axillary lymphadenopathy and breast examination was unremarkable. There were two sinus tracts discharging thick pus in the right hypochondrium and just above them was a non tender, fluctuant swelling with reddish blue discoloration of the overlying skin. An early pus point was forming over this swelling (fig 2). Chest examination revealed coarse crepitations in right upper and mid chest. On examination of the oral cavity there were multiple dental carries and poor oral hygiene. Rest of the systems were normal.

Large trunk and breast abscesses were located on ultrasosnography; two hundred ml of pus was drained and sent for Gram stain, Acid Fast Bacilli smear, routine and TB culture. She was smear negative but had a non-homogenous infiltrate in the right upper and mid zones on the chest X-ray (fig 3). Erythrocyte Sedimentation Rate was 60 mm and there was mild normocytic anemia. Other blood chemistries were normal.

On the basis of the history of chronic cough, cold abscesses on

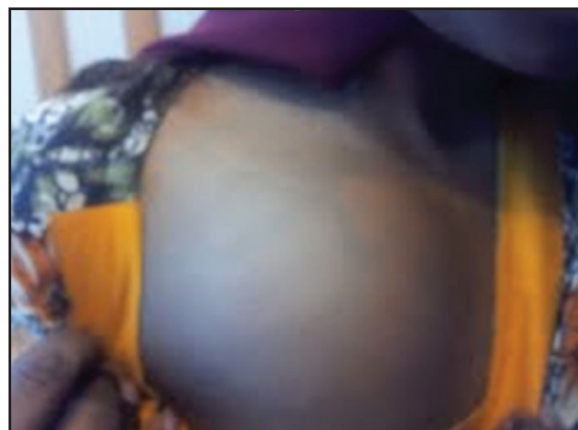


Fig 1: Large breast abscess (Permitted by the patient)



Fig 2: Draining sinus and swelling in right hypochondrium

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Fig 3: Non Homogenous Infiltrates in right chest

the trunk and upper chest and infiltrates on chest X-ray a provisional diagnosis of tuberculosis was made and she was started as “New Tuberculosis” on Anti Tuberculosis Therapy (ATT) on 6/3/12. Her right breast was completely drained under ultrasound guidance. However, when she returned for follow up two weeks later, a tense abscess had reformed. The trunk abscess had also discharged through a sinus (fig 4). She was had cough with weight loss.

The pus from the breast abscess was thick and cloudy with a few tiny yellow granules. It was inoculated on blood agar (Oxoid, UK), MacConkey’s agar (Oxoid, UK), chocolate agar and Sabouraud’s Dextrose Agar (Oxoid, UK), and incubated at requisite temperatures. Growth appeared within 48 h on blood agar with round, smooth, convex and chalky white colonies (fig 5). Morphology showed Gram positive filamentous rods (fig 6) which remained unstained by modified Ziehl-Neelsen technique; and were catalase positive and Aesculin and urease negative. They were identified as aerobic



Fig 4: Sinuses over trunk (exuded thick pus)

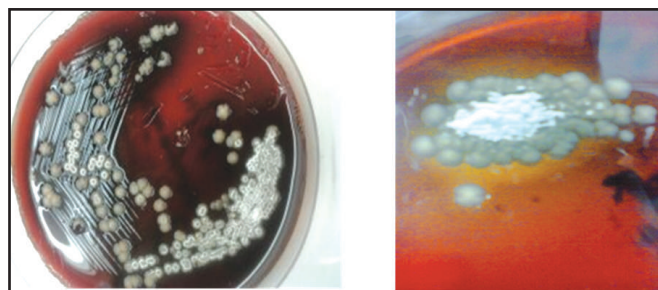


Fig 5: Convex, chalky white colonies on blood agar

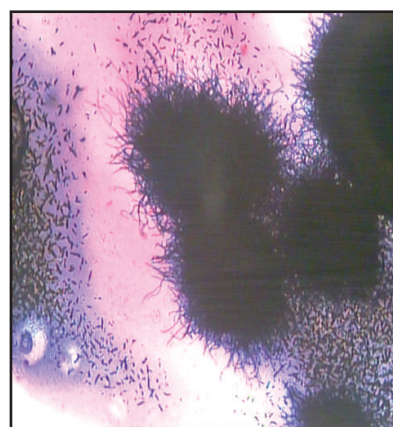


Fig 6 : Gram positive filamentous rods as seen on morphology

actinomycetes species. Sensitivity testing was carried out by disc diffusion method which showed that the isolate was sensitive to amoxycillin-clavulanic acid, ciprofloxacin, amikacin and linezolid; and resistant to cefotaxime, imipenem and co-trimoxazole.

The patient was diagnosed as having actinomycosis of lungs, chest and abdominal wall soft tissue. ATT was stopped and admission for parenteral antibiotics was advised but as the patient refused admission she was put on per oral linezolid 600 mg twice daily. The response to the treatment has been favourable and is being followed.

Discussion

Actinomycetes are Gram-positive bacteria; non-acid fast, non-spore forming, anaerobic or microaerophilic and arranged as branching filaments.⁶ A morphologically similar group - the aerobic actinomycetes include unusual human pathogens which are ubiquitous in nature and can cause debilitating disease in both immune-competent and immunocompromised hosts.⁷ Their taxonomic classification is evolving and includes *Actinomadura*, *Rhodococcus* spp, *Gordona* spp, *Dermatophilus congolensis*, *Streptomyces* spp, *Thermophilic actinomycetes*, *Nocardia* spp, *Micromonospora* spp and *A. orientalis*.^{6,7} The bacteria in our case grew aerobically; exact species could not be identified due to laboratory constraints.

Actinomycosis is a rare but curable infection. It has been called a masquerader as it pretenses tuberculosis, fungal infection or malignancy and can be challenging to diagnose even with experienced clinicians.⁸ A breach in the mucosal barrier or presence of devitalized tissue are prerequisites for causing infection in these otherwise low virulent commensals. Dental caries or manipulations and oromaxillofacial trauma are the most common triggering events.⁷⁻⁹ In our case, the patient had poor oral hygiene and multiple dental caries which might have been a trigger for infection. Actinomycosis is slowly destructive with a predilection for the jaw, lung, ileocecal junction and pelvis. Thus the main clinical types as classified by Cope in 1938 are orocervicofacial (50%), pulmono-thoracic (30%) and abdomino-pelvic (20%) actinomycosis respectively.¹⁰ Hematogenous spread to liver and central nervous system has also been reported.⁸

Symptoms of soft tissue disease are often non-specific: low grade pyrexia occurs in >50% of patients.^{11,12} Pain is rare and mostly there is a sensation of superficial tension around the forming mass or abscess. Abscesses can burst forming draining sinuses which are seen in approximately 40% of cases, and when present, may be helpful in narrowing the differential diagnosis.¹³ In our case, presence of multiple draining sinuses was quite suggestive of actinomycosis.

The history of low grade fever, weight loss, chronic cough, chest pain and radiographic features of pulmonary actinomycosis closely resemble those of lung cancer and chronic granulomatous pulmonary infections such as tuberculosis and fungal infections.⁵ Therefore, patients can be misdiagnosed and treated as tuberculosis. Weese and Smith reported that the infection was only suspected in 10% of patients admitted with chronic chest symptoms and average time from symptom onset to definitive diagnosis was estimated to be 6 months.¹¹ In our case, the patient was initially diagnosed as disseminated tuberculosis and started on ATT before the lab isolated aerobic actinomycetes.

In tissues, *actinomycetes species* form filamentous clusters surrounded by a predominantly neutrophilic infiltrate. When these clusters exude from soft tissues through sinus tracts they are macroscopically visible yellowish coloured particles called "sulfur granules". On Haematoxylin Eosin staining, they appear pink as they are surrounded by a eosinophilic proteinaceous coating which partly represents an ill-defined host response and partly eosinophil granule major basic protein.⁶ However, sulfur granules are not pathognomonic as they have been found in other infections such as nocardiosis and botryomycosis.^{13,14}

Actinomyces have been isolated from tissues in combination with other co-pathogens like *Enterobacteriaceae*, *Fusobacterium*, *Bacteroides*, *Staphylococcus* and *Streptococcus* spp which enhance their virulence potential.¹⁵ Even when the clinician suspects actinomycosis on the basis of recurrent

abscesses, sinus tracts, relapsing infection and thick lumpy pus, the definitive diagnosis can be very difficult and is only established by direct identification of the organism from sulfur granules or other acceptable clinical specimen like pus or invasive biopsy material.⁸ However, Actinomycetes growth is very difficult even on appropriate media (recovery rates from culture are < 50%).¹⁶ Moreover, identification of many of the diverse species constituting the aerobic actinomycetes is often very difficult in a microbiological setting.⁷ The macroscopic presence of the classic sulfur granules in tissue specimens or pus may prove useful when diagnosing, even if these features are not pathognomonic.^{12,15} In the present case, clinical suspicion was aroused when sulfur granules were seen on naked eye examination of the pus specimen.

For anaerobic actinomycetes an extended course of high dose penicillin G remains the cornerstone of treatment.⁵ The aerobic actinomycetes are usually resistant to ampicillin and the standardization of the antimicrobial susceptibility methods is not well defined.⁷ The Clinical and Laboratory Standard Institute (CLSI) recommends susceptibility testing of amikacin, amoxicillin-clavulanic acid, ceftriaxone, clarithromycin, imipenem, linezolid, co-trimoxazole and minocycline.¹⁷ Drug susceptibility testing for aerobic actinomycetes is useful not only in selecting the initial antimicrobial regimen but also for monitoring development of resistance which may complicate the course of treatment. Prolonged therapy beyond the resolution of measurable disease is needed to prevent relapse.⁷ Our isolate was sensitive to amoxicillin-clavulanic acid, linezolid, amikacin and ciprofloxacin and resistant to ampicillin, cephalosporins and imipenem.

Actinomycosis is considered an important clinical entity as it is difficult to diagnose, frequently misdiagnosed as tuberculosis, requires long term antibiotic therapy but has a good prognosis if diagnosed early and treated adequately.¹⁸⁻²¹

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Upcoming International Events – Infectious Diseases

July 2012

- 6th** 1st Asia Pacific Clinical Epidemiology and Evidence Based Medicine Conference Kuala Lumpur, Malaysia
- 7th** Infectious Disease Review Seattle, United States of America
- 12th** 6th Ditan International Conference on Infectious Diseases, Beijing, China

August 2012

- 9th** The 39th Annual Scientific Meeting of the Infectious Diseases Society for Obstetrics and Gynecology Whistler, Canada
- 25th** 2012 Infectious Disease Board Review Course McLean, USA

September 2012

- 2nd** Infectious Disease Shanghai, China
- 7th** The Viral Hepatitis Congress Frankfurt, Germany
- 15th** Modern principles of treatment of neuro-oncology diseases. Prospects for functional neurosurgery Yalta, Ukraine

October 2012

- 15th** The 4th Biennial Conference of the International Association for Ecology and Health: Sustaining Ecosystems, Supporting Health Kunming City, China
- 19th** ABSA 55th Annual Biological Safety Conference, Orlando, Florida, USA
- 27th** 3rd International Conference on Stem Cells and Cancer (ICSCC-2012): Proliferation, Differentiation and Apoptosis New Delhi, India

November 2012

- 10th** Infectious Disease Honolulu, USA
- 19th** Internal Medicine Review and Update: Inpatient and Outpatient Care for the Hospitalist and PCP-2012

Sarasota, USA

- 21st** II International Conference on Antimicrobial Research (ICAR2012) Lisbon, Portugal
- 28th** The 6th Forum on Respiratory Tract Infections Barcelona, Spain

December 2012

- 10th** Pediatric Emergency Medicine: Emergent and Urgent Challenges-2012 Sarasota, USA
- 23rd** Infectious Disease Conference Depart Ft. Lauderdale, USA
- 24th** Infectious Diseases in Adult Patient: A Primary Care Update-2012 Sarasota, USA

January 2013

- 14th** Pediatric Infectious Diseases: An Evidence-Based Approach-2013 Sarasota, USA

February 2013

- 11th** Family Medicine: An Evidence-Based Approach to Patient Care-2013 Sarasota, USA
- 18th** Emergency Medicine: An Evidence-Based Approach to Adult Care-2013 Sarasota, USA

March 2013

- 11th** Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings-2013 Sarasota, USA
- 17th** Infectious Disease Review for the Primary Care Physician Departs Ft. Lauderdale, USA

April 2013

- 15th** Emergency Medicine: Practicing According to the Evidence-2013 Sarasota, USA

November 2013

- 20th** 8th World Congress of the World Society for Pediatric Infectious Diseases, Cape Town, South Africa

Instructions to Authors

Scope

The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJ). The Journal accepts Original Articles, Review Articles, Brief Reports, Case Reports, Short Communications, Letter to the Editor and Notes and News in the fields of microbiology, infectious diseases, public health; with laboratory, clinical, or epidemiological aspects.

Criteria for publication

All articles are peer reviewed by the IDSP panel of reviewers. After that the article is submitted to the Editorial Board. Authors may submit names and contact information of 2 persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

Submission of the Manuscript

Manuscripts must be formatted according to submission guidelines given below, which are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (originally published in *N Engl J Med* 1997;336:309-15). The complete document appears at www.icmje.org. Please submit one complete copy of the manuscript and all enclosures to **The Managing Editor, Infectious Diseases Journal of Pakistan, Department of Pathology and Microbiology, The Aga Khan University, Stadium Road, P.O. Box 3500, Karachi 74800, Pakistan**. An electronic copy of the manuscript must also be sent to maahin1@yahoo.com and pak_idj@yahoo.com. All manuscripts submitted to IDJP must be accompanied by an Authorship Declaration stating that *'The authors confirm that the manuscript, the title of which is given, is original and has not been submitted elsewhere. Each author acknowledges that he/she has contributed in a substantial way to the work described in the manuscript and its preparation'*. Upon submission a manuscript number will be assigned which should be used for all correspondence.

Manuscript Categories

I. Original Articles

Articles should report original work in the fields of microbiology, infectious disease or public health. The word limit for original articles is 2000.

Title page

This should list the (i) title of the article, (ii) the full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. (iii) the corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. (iv) a short running title of not more than 40 characters (count letters and spaces) placed at the foot end of the title page. (v) a conflict of interest statement should also be included in this section

Abstract

Abstract should not exceed 250 words and must be structured in to separate sections headed *Background, Methods, Results and Conclusions*.

Please do not use abbreviations or cite references in the abstract. A short list of four to five key words should be provided to facilitate.

Background

The section must clearly state the background to the research and its aims. Controversies in the field should be mentioned. The key aspects of the literature should be reviewed focusing on why the study was necessary and what additional contribution will it make to the already existing knowledge in that field of study. The section should end with a very brief statement of the aims of the article.

Materials and Methods

Please provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. The design of study and details of interventions used must be clearly described. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration. All research carried out on humans must be in compliance with the *Helsinki Declaration*, and animal studies must follow internationally recognized guidelines. The authors are expected to include a statement to this effect in the Methods section of the manuscript. A description of the sample size calculation and statistical analysis used should be provided.

Results

Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of 5 illustrations (in a combination of figures and tables) per article. The results should be in past tense and repetition of results presented in the tables should be avoided. Exact *P*-values should be reported along with reporting of OR and RR with their Confidence Intervals where applicable.

Discussion

Emphasize the new and important aspects of the study and conclusions that follow from them. Do not repeat the details from the results section. Discuss the implications of the findings and the strengths and limitations of the study. Link the conclusions with the goals of the study but avoid unqualified statements and conclusion not completely supported by your data.

Acknowledgments

Acknowledge any sources of support, in the form of grants, equipment or technical assistance. The source of funding (if any) for the study should be stated in this section. Please see below for **format of References, Figures and Tables**.

II. Review Articles

Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. It should consist of critical overview of existing literature along with reference to new developments in that field. These should be comprehensive and fully referenced. Articles should contain an Abstract; Main Text divided into sections, Conclusions and References.

III. Brief Reports

Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references.

IV. Case Reports

Instructive cases with a message are published as case reports. Routine syndromes or rare entities without unusual or new features are invariably rejected. The text should contain no more than 1000 words, two illustrations or tables and up to 10 references. The authorship should not exceed 3-4 persons.

V. Letter to the Editor

These may relate to material published in the IDJP, topic of interest pertaining to infectious diseases, and/or unusual clinical observations. A letter should not be more than 300 words, one figure and 3-5 references.

VI. News and Views

Informative, breaking news updates in infectious diseases from around the world (approx. 200 words).

VII. Notices

Announcements of conferences, symposia or meetings may be sent for publication at least 12 weeks in advance of the meeting date. Details of programs should not be included.

References

Number references consecutively in the order in which they are first mentioned in the text. Identify references in text, tables and legends by Arabic numerals (in superscript). References cited only in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification of the particular table or illustration. Bibliography should be given in order. Authors, complete title, journal name (Abbr), year, vol, issue, page numbers. According to "Uniform

Requirements of Manuscripts submitted to Biomedical Journals", as cited in N Engl J Med 1997; 336:309-15.

Tables and Figures

Data reported either in a table or in a figure should be illustrative of information reported in the text, but should not be redundant with the text. Each table must be presented on a separate sheet of paper and numbered in order of appearance in the text. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes. Results which can be described as short statements within the text should not be presented as figures or tables.

Illustrations

Illustrations should be numbered, given suitable legends and marked lightly on the back with the author's name and the top edge indicated. Original drawings may be submitted although high quality glossy photographs are preferable. They should be kept separate from the text. If possible, figures should be submitted in electronic format as either a TIFF (tagged image file format) or JPEG format. Minimum resolution for scanned artwork is:

- ❖ Black & white line illustration (e.g. graphs): 600 dpi
- ❖ Black & white halftone illustrations (e.g. photographs): 300 dpi
- ❖ Color illustrations: 400 dpi (note that color images should be split CMYK not RGB)

Plagiarism

Authors should refrain from plagiarism and should double check their work before submitting it for publication. Adequate references should be provided for text from other sources.

Authorship Criteria

Those who have contributed sufficiently to the conceptualization, design, collection and analysis of data and writing of the manuscript should be granted authorship. Ideally all authors should be from the same department except for studies that are multi center or multispecialty.

Instructions updated - April 2012.

Editor IDJ

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