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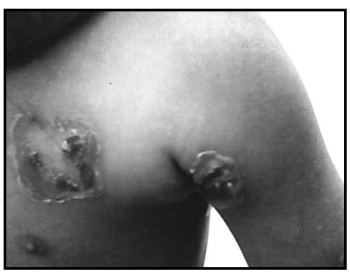

Chickenpox crust on chest and arm of a child

Courtesy: Department of Community Medicine, Liaquat University of Medical & Health Sciences, Jamshoro.

EDITORIAL

Biosafety and Pakistan

'Biosafety' pertains to protection of personnel working in the laboratories dealing with infectious agents. There is always a possibility of catching infection while working with micro-organisms if proper protocols are not followed. Laboratory associated infections (LAIs) are well documented all over the world even at the best possible set-ups, however, data is lacking in our country. The related problems are compounded by emerging and re-emerging infections along with the latest developments like living modified organisms (LMOs) and genetically modified organisms (GMOs).

Closely related to biosafety is 'biosecurity' which implies secured storage of micro-organisms so that they may not fall into precarious hands for any malicious use. This requires risk assessment and formulation of standing operating procedures along with strict implementation. There has been significant importance imparted to these two subjects in the recent past especially in the light of prevailing scenarios.

Various guidelines are available at the international level that clearly elaborate the safety methodology in detail like World Health Organization (WHO) guidelines.[1] Biosafety in Microbiological & Biomedical Laboratories (BMBL) is considered to be the bible for biosafety matters.[2] These meticulously elaborate the various biosafety levels and accordingly the required protocols and controls. Cartagena Protocol provided the fibre by highlighting the role of biosafety in scientific research.[3] Similarly, Biological and Toxin Weapon Convention (BTWC) at the United Nations has helped forming a platform whereby 163 states are party to the Convention.[4] This Convention has opened the opportunities for bringing the state parties to meaningful dialogue thus facilitating the national and international efforts.

The efforts at our national level have been noteworthy which in turn depict the affirmative posture of the Government of Pakistan. The commitment has been highly appreciated at the international level. Some of the salient contributions include:

1. National Focal Point for the Biological & Toxin Weapon Convention (BTWC) has been appointed at Ministry of Foreign Affairs (MoFA), Pakistan; Director General Disarmament has been delegated this challenging task. Over the last couple of years there has been tremendous boost to the national integration and policy making due to untiring efforts of Dr Irfan Yusuf Shami, DG Disarmament, and his team.
2. Formation of 'Interagency Working Group on Regulation of Bioscience & Technology', under Disarmament Division, MoFA, thus bringing all the stakeholders over single platform. These include important concerned ministries and institutes. Under this important contributions are:
   a. Drafting and approval of legislation on BTWC.
   b. Code of Conduct for Life Scientists.
   c. International collaborative efforts.
3. Biosafety Rules and Biosafety Guidelines were introduced in 2005.[5,6] The rules highlight micro-organisms and GMOs with regards to manufacture, storage and transfer at research and diagnostic institutes or laboratories at any level.
4. National Biosafety Committee (NBC) funtions under the supervision of Secretary Ministry of Environments. It has provincial representation along with representatives from Ministry of Health, Ministry of Science & Technology, Ministry of Food & Agriculture, Ministry of Education, Pakistan Agriculture Research Council, Department of Plant Protection and Environmental Protection Agency. Technical Advisory Committee, chaired by Director General EPA, works under the NBC for implementation of various tasks.
5. Institutional Biosafety Committees are being strengthened at the concerned institutes for looking after the biosafety issues at local level. Emphasis is being given to oversight of research especially related to infectious agents.
6. Some time back, Higher Education Commission (HEC) of Pakistan formed National Core Group in Life Sciences (NCGLS) comprising life scientists from related disciplines. NCGLS established four resource centres at various universities and organized national and international training activities. Dr Anwar Nasim, Advisor Organization of Islamic Countries for Science & Technology (COMSTECH), has been the main motivational force behind these activities.
7. National Commission on Biotechnology (NCB) was established in 2001 for strengthening research and collaboration. In 2003, NCB launched Pakistan National Policy & Action Plan on biotechnology.
8. International Council of Life Scientists has established Pakistan Chapter, through dedicated efforts of Dr Anwar Nasim, for collaboration in the biotechnology related affairs. A number of workshops have been organized in different cities of the country.
9. Pakistan Biological Safety Association (PBSA) was established in March 2008 with a small group of dedicated professionals.[7] Among the founder members are biosafety experts like Dr Erum Khan, Dr Aamer Ikram and Dr Shamsul Arfin Qasmi. Over a couple of years, it has representation from all over the country. The main objectives have been awareness raising and

capacity building in biosafety. A large number of workshops and short courses have been organized at different cities. Efforts of PBSA have been appreciated at international level. PBSA has been accepted as member organization of International Federation of Biological Associations (IFBA).

10. University curricula for biosafety have been developed as part of concerned specialty training. The subject training has been launched at two elite universities in the country. The training has been made integral part of the concerned specialties and the future plan is to have it as separate field.
11. General awareness is being enhanced among the concerned at each and every possible occasion. 'Pathology Week' is celebrated every year from 2nd May to 8th May. This opportunity is taken to raise awareness among the laboratory workers especially young technicians.

The areas that still need particular attention are availability and proper usage of biosafety cabinets and personal protective equipment, frequent awareness opportunities for laboratory staff, more training regarding transportation of infectious material according to international standards, proper certification system for biosafety cabinets, body for recognition or authentication of expertise in biosafety field and microbiology laboratory accreditation system.

To conclude, a comprehensive approach has to be implemented for enforcing biosafety and biosecurity in the laboratories through proper guidelines, strict implementation of rules and regulations, increasing awareness and training and vigilant oversight mechanism.

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In Vitro Efficacy of Piperacillin/Sulbactam (Combicin) Against Gram Negative Rods Isolated from Various Clinical Specimens

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Background and objective
Choice of appropriate antimicrobial agent in treatment of infections is a major problem. Gram negative isolates are notorious for rapid development of resistance. Since the susceptibility profile of various isolates changes rapidly, knowledge about the efficacy of newly introduced drugs is important for clinicians. The objective of this study was to determine the *in vitro* efficacy of piperacillin/sulbactam (combicin), a beta lactam/beta lactamase inhibitor combination against gram negative rods isolated from various clinical specimens at Armed Forces Institute of Pathology Rawalpindi.

Method
A total of 497 consecutively isolated Gram negative rods from various clinical samples submitted at AFIP from June 2009 to October 2009 were included in the study. Antibiotic susceptibility of these isolates against piperacillin/sulbactam was carried out by disc diffusion method.

Results
Of the 497 isolates tested, 362 (72%) were sensitive, 130 (26%) were resistant and 5 (1%) were intermediately susceptible to piperacillin/sulbactam. The most resistant microorganism to this compound was *Klebsiella pnuemoniae*, as 20/56 (35%) isolates were resistant where as the most susceptible organism was *Proteus* spp, with 18/20 (90 %) isolates being susceptible to piperacillin/sulbactam.

Conclusion
The newly introduced agent piperacillin/sulbactam has good overall efficacy against the usual gram negative isolates in our setup. This antimicrobial, however, should be cautiously used as an empirical agent in seriously ill patients suspected of gram negative infections.

Key words
Antimicrobial Susceptibility, Beta-lactam, Beta-lactamase Inhibitors, Combicin, Piperacillin, Sulbactam.

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Introduction
Bacterial pathogens especially gram negative rods are becoming notorious for development of rapid antimicrobial resistance. There is growing trend in clinical practice to use higher generation antibiotics especially the beta-lactam group of antibiotics. This trend exerts a selective pressure in development of resistance against these agents. Multidrug resistant (MDR) isolates are commonly encountered especially in intensive care units. Another problem with these MDR isolates is that they are also resistant to other classes of antibiotics which further complicate treatment options in hospitals[1]. Since majority of antibiotics used in clinical practice are beta-lactams, this along with cross resistance to other agents have restricted their empirical use. Clinicians therefore must be aware of the current antibiotic resistance trends among the common bacterial isolates in our setup. One of the methods to combat resistance is to combine a beta-lactam with a beta-lactamase inhibitor[2]. This prevents the beta lactamases (one of the major mechanism of resistance developed by gram negative rods) from acting on the beta-lactam antibiotic.

This study is aimed to find the *in vitro* efficacy of newly introduced drug piperacillin/sulbactam (Combicin), against gram negative rods isolated from various clinical specimens at AFIP Rawalpindi.

Materials and methods
A total of 497 non duplicate gram negative rods isolated from various clinical samples submitted at Armed Forces Institute of Pathology Rawalpindi (AFIP) from June 2009 to October 2009 were included in the study. The samples consisted of blood, pus, urine, CSF, pleural fluid, sputum, bronchoalveolar lavage, catheter tips and body fluids etc. The samples were inoculated on respective culture media. Following 24 to 48 hrs incubation at 35 ± 2°C, the isolates were identified as Gram negative rods based on colony morphology, Gram staining, oxidase, catalase and motility tests. After preliminary identification, 0.5 McFarland (turbidity standard) bacterial suspensions were prepared. Using modified Kirby-Bauer disc diffusion technique lawn was made on Mueller Hinton agar (Mast diagnostics, UK) and antibiotic discs were applied according to CLSI guidelines[3]. A 130 µg antibiotic disc of piperacillin/sulbactam was also applied. Simultaneously the same suspension was used for identification of isolate by

inoculating on to sugar sets/ API test strips (BioMerieux, France). Final identification of the isolate was made on basis of biochemical profile. Susceptibility was recorded by interpretation of zone diameters according to manufacturer's guidelines (Table 1). *E. coli* ATCC 25922 was used as control strain.

Table 1: Susceptibility criteria – Zone diameters

Sensitive	Intermediate	Resistant
≥20 mm	18-19 mm	<18 mm

Results

Out of the total 497 isolates tested, 362 were susceptible, 130 were resistant and five were of intermediate susceptibility to piperacillin/sulbactam. Majority of the isolates were yielded from urine followed by pus and pus swabs. The susceptibility of piperacillin/sulbactam against various isolates is given in the table 2.

Discussion

Enterobacteriaceae and non-fermenting Gram-negative bacteria have emerged as an important nosocomial pathogens[4]. With the extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk wards, resistance to these drugs has become a major problem all over the world[1]. Among the β-lactams, third generation cephalosporins, such as ceftazidime, cefotaxime, and ceftriaxone are routinely used in our clinical setting, and resistance to these drugs, due to β-lactamase production, is rampant[5]. MDR mediated through R plasmids among gram-negative bacteria has become a major nosocomial problem worldwide[4]. Such MDR strains have been consistently reported in recent years from several setups across the globe[6,7]. Mortality, morbidity and cost of treatment have considerably risen because of these resistant isolates[8]. To date we have remained one step ahead of bacteria in developing newer and better antibiotics but we cannot continue to rely upon coming up with an alternative when resistance develops to one agent as risk of an outbreak by strains resistant to all known antibacterial agents is always there. Rational use of antibiotics and good collaboration between microbiologist and clinicians is therefore mandatory to prevent bacterial resistance. One of the methods to do this is by changing the antibiotic regime from time to time with different classes of antibiotics. This reduces the selective antibiotic pressure and piperacillin/sulbactam seems to be a good choice in this regard. In one of the study conducted by Srujana Mohanty *et al*,

Table 2: Antibiotic susceptibility of various organisms against piperacillin/sulbactam

Name of isolate	Total	Susceptible	Intermediate	Resistant	% susceptible
E. coli	220	155	03	62	70
P. aeruginosa	89	73	0	16	82
K. pnuemoniae	56	34	02	20	60
A. baumannii	36	25	0	11	69
A. johnsonii	19	15	0	04	79
Proteus spp	20	18	0	02	90
C. fruendii	11	08	0	03	73
K. oxytoca	08	05	0	03	62
Enterobacter spp	10	08	0	02	80
Morganella morgani	06	06	0	0	100
Serratia marcescens	10	04	0	06	40
Provedentia spp	05	05	0	0	100
Citrobacter diversus	02	02	0	0	100
Salmonella paratyphi	01	01	0	0	100
Stenotrophomonas maltophilia	03	02	0	01	67
Moraxella mansonii	01	01	0	0	100

Antimicrobial susceptibility of the five most common isolates revealed that 70% of *E. coli*, 82% of *P. aeruginosa*, 60% of *K. pnuemoniae*, 72% of *Acinetobacter* spp and 90% of *Proteus* spp were susceptible to piperacillin/sulbactam. Out of the top five isolates, most resistant was of *Klebsiella pnuemoniae* (35%). The most susceptible isolate was *Proteus* spp, with 18/20 (90 %) isolates being susceptible to piperacillin/sulbactam.

susceptibilities of the various beta lactam/beta lactamase inhibitor combinations including piperacillin/tazobactam, cefoperazone/sulbactam, and ticarcillin/clavulanic acid against gram negative rods was 81.37, 76.06 and 45.48 per cent respectively.[9] The results of our study imply that its susceptibility profile is comparable to piperacillin/tazobactam and cefoperazone/sulbactam. In another study conducted by Zhiyong *et al* in China the efficacy of piperacillin/sulbactam and piperacillin/tazobactum were 93.2% and 93.4% respectively against aerobic gram negative rods[10]. *In vitro* efficacy of piperacillin/sulbactam according to this study was significantly higher than ours presumably because it is an older study and the agent was newly introduced at that time. In another study, by Andreas Weber *et al* in 2008, the *in vitro* efficacy of piperacillin/sulbactam was 65% which is more or less comparable to our results[11].

Another encouraging finding of our study was the fact that 73% of Acinetobacter spp were susceptible to this compound. Keeping in view the recent MDR profile exhibited by *Acinetobacter* spp, piperacillin/sulbactam provides a good alternative to manage such MDR isolates. These results are in concordance with one of the study carried out by Higgins *et al*, reporting 80.9% of *A. baumannii* isolates susceptible to piperacillin/sulbactam whereas tazobactum/piperacillin was effective in 73.9% cases of isolates. These results are quite encouraging as CLSI recommends ampicillin/sulbactam as the only compound with beta-lactam/beta-lactamase inhibitor combinations as one of the primary antimicrobial to be tested and reported for *Acinetobacter* spp. Further comparative studies in our setup needs to be done to see if this combination is comparable in efficacy to ampicillin/sulbactam against MDR Acinetobacter isolates.

In addition to *Acinetobacter* spp, our study has revealed that this compound is also quite effective against *E. coli*, *Pseudomonas aeruginosa*, *K. pneumoniae*, and *Proteus* spp. So this combination could serve as a ray of hope as one of the alternative antimicrobial to combat life threatening infections caused by these microorganisms.

Conclusion

Piperacillin/sulbactam showed good overall efficacy against the Gram negative isolates in our setup. Nonetheless it should be used cautiously as an empirical agent in treating suspected Gram negative infections especially in intensive care settings.

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Microorganisms in Burn Wounds

Mohammed Nayef AL-Bdour, Lamees Arabiyat, Maher Alkatib, Khaled Almaytah, Waleed Haddadin

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Abstract

Introduction
Infection of burn wounds continues to be the main cause of morbidity and mortality in patients with major thermal injuries admitted to hospitals. Infection causes 50 to 60% of deaths among burn patients in spite of all efforts. Set up for prevention starts from burn unit establishment to intensive therapy with antibiotics both topically as well as intravenous.

Aim
To analyze the isolation pattern and antibiotics sensitivity pattern of microorganisms yielded from swabs taken from burn wounds.

Materials and methods
This retrospective study was carried out at Burn Unit of King Hussein Medical Center from February 2009 to February 2010. A total of 78 burn wound swab samples from admitted patients were dealt with analysis of antibiotics sensitivity.

Results
Growth was yielded in 66 (84.6%) and *Pseudomonas aeruginosa* was the predominant organism in our setup (43.6%), followed by *Acinetobacter* spp (10.2%) and *Klebsiella pneumoniae* (10.2%). The age of patients ranged from 2-64 years.

Conclusion
Every burn unit should have its own microbiology data and antibiotics sensitivity, updated regularly in order to decrease the cost of management as well as both morbidity and mortality of the patients.

Key Words
Burns, Burn Ward, Burn Wound.

Introduction
Infection in the burn wound continues to be the main cause of morbidity and mortality in patients admitted to hospital with major thermal injuries. Infection causes 50% to 60% of deaths in burn patients in spite of intensive therapy with antibiotics both topically as well as intravenous, studying the microorganism prevalence in burn wounds with antibiotics sensitivity is essential in working strategy of burn centers.[1] This study was carried to analyze the isolation and sensitivity pattern of microorganism from the burn unit at Kings Hussein Medical Center.

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Materials and Methods
This retrospective study was performed in the burn unit of King Hussein Medical Center from February 2009 to February 2010. Burn wound swab cultures for patients admitted to burn unit were analyzed. Wound management in these patients consisted of daily washing and twice daily dressing with silver sulphadiazine cream or nitrofurazone ointment depending on burn depth and eschar formation.

Swab cultures were taken routinely twice weekly after cleansing of applied ointments, and at admission for all patients referred from other hospitals or if there was clinical indication such as fever or wound discharge. The wounds were swabbed and cultured as follows: A sterile cotton swab, moistened with sterile normal saline, was gently rubbed onto the burn wound surface. In laboratory the samples were dealt as per routine protocol. Samples were inoculated on Blood Agar (Oxoid), MacConkeys's Agar (Oxoid), Chocolate Agar, and Sabouraud's Agar as per the requirements. They were identified by the appearance of their colonies and results of biochemical tests. Sensitivity was done by disc diffusion method (Kirby-Bauer) using CLSI standards.

Results
Data showed that age of patients ranged between 2-64 years with male predominance (66%). Cultures yield was 84.6%. *Pseudomonas aeruginosa* was the predominant organism in our setup (56%), followed by *Acinetobacter* spp (12.1%) and *Klebsiella* spp (12.1%), least predominant organisms were *enterococci* (1.5%) and *Candida albicans* (1.5%) (Fig 1).

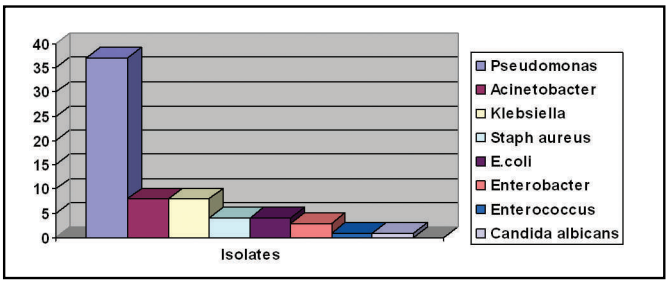

Figure 1: Isolation pattern of organisms (n=66)

Table 1: Antibiotic sensitivity of the three dominant microorganisms

Micro-organism	Antibiotics							
	Imipenem	Amikacin	Tazobactam	Aztreonam	Ceftazidime	Cefepime	Cipro floxacin	Genta micin
Pseudomonas aeruginosa	*64%*	*41%*	*44%*	*19%*	*17%*	*19%*	*53%*	*23%*
Acinetobacter spp	*50%*	*25%*	*25%*	*25%*	*25%*	*25%*	*37.5%*	*37.5%*
Klebsiella spp	*75%*	*12.5%*	*62.5%*	*0%*	*0%*	*0%*	*87.5%*	*0%*

Discussion

Burn wounds colonization and ultimately infection remains a major challenge for all burn units worldwide and affects the quality of treatment and patient prognosis. It can jeopardize any attempt of cosmetic as well as functional treatment, e.g. can compromise grafting procedures and subsequently increase in scars and contractions. According to previous study done in same burn unit, the mortality rate for major burns was 29% with no deaths due to infections in minor and moderate burns.[1]

It has been estimated that 75% of all deaths in burns exceeding 40% of total body surface area are related to infection.[2] Initially, the burnt area is considered free of microbial contamination, but gram-positive bacteria in the depth of sweat glands and hair follicles heavily colonize the wounds within 48 hrs of the injury.[3,4] Topical antimicrobials decrease microbial overgrowth but seldom prevent further colonization with other potentially invasive bacteria and fungi. These are derived from the patient's gastrointestinal and upper respiratory tract and hospital environment.[5,6]

Following colonization, these organisms penetrate viable tissue depending on their invasive capacity, local wound factors and the degree of the patient's immunosuppression.[6] If deeper tissue is invaded, disseminated infection is likely to occur.[4] Emphasis must therefore be on early identification of local signs of invasive burn wound infection.

The causative infective microorganisms in any burn facility change with time.[7,8] Individual organisms are introduced into the burns ward by the wounds of new patients. These organisms then persist as the resident flora of the burn treatment facility for a variable period of time, only to be replaced later by newly arriving microorganisms. Introduction of new topical agents and systemic antibiotics influence the flora of the wound.[7,8]

Pseudomonas aeruginosa remains the predominant isolate from the burns wards. In our study it was followed by *Acinetobacter* spp. The problem with the later species is that it is rapidly acquiring resistant to most of the available antimicrobial agents. This would definitely lead to treatment failure in otherwise complicated burn cases.

It is just not sufficient to be aware of the microorganisms that pose a problem for burn patients. To have an in-depth knowledge of the organisms that are predominant in that particular treatment facility during the particular period along with their sensitivity pattern is vital as many septic burn patients need to be treated with antibiotics before the results of microbiological cultures are available. This would be crucial to reduce the overall infection-related morbidity and mortality.

Conclusion

Every burn unit should have its own microbiology data and antibiotics sensitivity pattern, updated regularly in order to decrease the cost of management as well as morbidity and mortality. This in turn is crucial measure for infection control.

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ORIGINAL ARTICLE

Spectrum and Antimicrobial Susceptibility Pattern of Pathogens Causing Urinary Tract Infection-Experience in a Tertiary Care Setting

Alina Amjad, Irfan Ali Mirza, Shahid Ahmad Abbasi, Umme Farwa, Azfar Sattar, Zeeshan Ahmed Qureshi

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Abstract

Objective
To determine the spectrum and antimicrobial susceptibility pattern of pathogens causing urinary tract infection from the samples received at AFIP Rawalpindi.

Place and duration of study
The study was carried out at Department of Microbiology, Armed Forces Institute of Pathology from January 2010 to June 2010.

Material and method
This was descriptive cross-sectional study. A total of 500 bacterial isolates out of 2050 urine culture samples, for a period of six months were included in the study. Identification was carried out by standard biochemical profile of the organisms. The antimicrobial susceptibilities of isolated organisms were performed by disk diffusion method as recommended by Clinical Laboratory Standard Institute.

Results
Out of the culture positive cases, gram-negative bacteria constituted the largest group with a total of 434 (87%) isolates, while gram-positive bacteria constituted only 44 (8.8%) isolates. *E. coli* was the most common isolate accounting for 63% of the total positive bacterial cultures followed by *Klebsiella* spp (9%) and *P. aeruginosa* (7%). Susceptibility pattern of *E. coli* revealed that 96% of isolates were sensitive to imipenem, 91% to piperacillin-tazobactam and 87% to nitrofurantoin. For *Klebsiella* spp, 86% of isolates were susceptible to imipenem and 71% to piperacillin-tazobactam. As regards *Acinetobacter* spp, 94% of isolates were susceptible to piperacillin-tazobactam and 70% to imipenem and piperacillin-sulbactam. Antibiogram of *P. aeruginosa* revealed that 94% of isolates were sensitive to ceftazidime and 86% to imipenem. Nitrofurantoin was the most effective urinary antibiotic in case of *Enterococcus* spp as 85% of isolates were susceptible to this compound.

Conclusion
From the present study we conclude that *E. coli* was the predominant urinary pathogen in our set up. The antimicrobial susceptibility patterns of *E. coli* revealed that majority of isolates were susceptible to imipenem and piperacillin-tazobactam while susceptibilities to ampicillin, cotrimoxazole and ciprofloxacin were very low. Majority of *E. coli* and *Enterococcus* spp isolates were susceptible to nitrofurantoin.

Key words
Antimicrobial Susceptibility, Disc Diffusion Method, Urinary Tract Infection.

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Introduction

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. It is the second most common type of infection in the body. UTIs account for about 8.3 million hospital visits each year.[1] Women are especially prone to UTIs for reasons that are not yet well understood. One woman out of five develops a UTI during her lifetime.[2] UTIs in men are not as common as in women but can be very serious when they do occur. UTIs encompass a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urethritis.

Virulence factors related to urinary isolates promote adherence to mucosal surfaces leading to subsequent infections. Host factors such as the epithelial cell receptivity, are also important in the infective process. Although fungi and viruses are occasional etiological agents, UTIs are predominantly caused by bacteria which include *Escherichia coli*, *Pseudomonas* spp, *Streptococcus* spp, *Proteus* spp, *Klebsiella* spp, *Staphylococcus* spp, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Candida* spp and *Mycoplasma*.[3] Extremes of ages, female gender, pregnancy, instrumentation, neurologic dysfunction, renal disease, and expression of A, B and H blood group oligosaccharides on the surface of epithelial cells are considered predisposing factors for the development of UTIs.[4]

UTI constitute a great proportion of diseases requiring antibiotics. Shorter duration of antibiotic treatment as well as treatment administered without considering the microbiologic and antimicrobial susceptibility data results in resistance among uropathogens. There has been a steady increase in the level of antimicrobial resistance to commonly used antibiotics amongst the UTI causing bacterial pathogens.[5]

Knowledge of etiological agents and their antimicrobial sensitivities to available drugs is of immense value to the rational selection of antimicrobial agents in treating UTI. This study was thus aimed to find out spectrum and antimicrobial susceptibility pattern of pathogens causing urinary tract infection in our setup.

Materials and methods

This laboratory based study was carried out in the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from January 2010 to June 2010. Freshly voided midstream urine specimens were collected aseptically from patients suspected of having UTI and hospitalized in different wards as well as patients referred to our facility from the outpatient department.

Inoculation of urine in the laboratory was carried out as soon as possible. If some delay was expected in processing, the specimens were refrigerated. Culture of urine was carried out using the semi-quantitative strip method (MAST Bacteruritest) on Cysteine Lactose Electrolyte-Deficient agar (CLED). After the inoculation, the culture plate was incubated aerobically with 5-10% CO_2 at 37°C for 24 hrs. In case significant growth (> 10^5 CFU/ml i.e., 20 CFU) was obtained, identification of the microorganisms was done with the help of colony appearance, Gram staining, catalase production, coagulase test, oxidase test, motility, biochemical profile and serology if required. API-20E or API-20 NE galleries (BioMerieux, France) were utilized for the biochemical identification of lactose fementers or non-lactose fermenters respectively.[6]

Antibacterial susceptibility testing of the isolates was done using the Kirby-Bauer disk diffusion method following the definition of the Clinical and Laboratory Standards Institute (CLSI 2010) using antibiotics containing discs from OXOID. Antibacterial susceptibility testing of the isolates was done on Mueller Hinton agar (OXOID, UK). Commercially available standard antibiotic discs of standardized concentrations were positioned at appropriate distances on the bacterial lawns and incubated at 37°C for 24 hours. The growth inhibition zones were carefully measured and recorded according to the standard Kirby-Bauer disc diffusion method and CLSI guidelines 2010.[7,8] An isolate was considered multi-drug resistant if it was resistant to at least three groups of the antibiotics tested.

The antibiotics discs and the concentration used were amikacin 30µg, imipenem 10µg, nitrofurantoin 300µg, piperacillin-tazobactam 105µg, gentamicin 30µg, ceftriaxone 30µg, ceftazidime 30µg, amoxicillin- clavulanic acid 30µg, ciprofloxacin 5µg, cotrimoxazole 25µg, ampicillin 25µg, vancomycin 30µg, linezolid 30µg, teicoplanin 30µg, sulbactam-cefoperazone 105µg and piperacillin-sulbactam 130µg.

Isolates were classified as either resistant, intermediate sensitive or sensitive based on the CLSI. *S. aureus* (ATCC25923), *Enterococcus faecalis* (ATCC51209), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC27853) were used as control strains.

Results

Out of total 2050 urine specimens included in the study, 500 urinary samples yielded significant bacterial growth. Out of positive urine cultures, 255 (51%) were from females and 245 (49%) from males. Gram negative bacteria constituted the largest group with a total of 434 (87%) isolates, while Gram positive bacteria constituted only 44 (8.8%) isolates. *E. coli* (63%), *Klebsiella* spp (9%), *P. aeruginosa* (7%), together accounted for 79% of the total number of culture positive isolates (Fig 1). Susceptibility pattern of *E. coli* revealed that 96% of isolates were sensitive to imipenem, 91% to piperacillin-tazobactam, 82% to nitrofurantoin, 81% to amikacin (Table 1). The antimicrobial susceptibility pattern of members of Enterobacteriaceae (other than *E. coli*), *P. aeruginosa*, *Acinetobacter* spp, *Staphylococcus* spp and *Enterococcus* spp are shown in tables 2, 3 and 4.

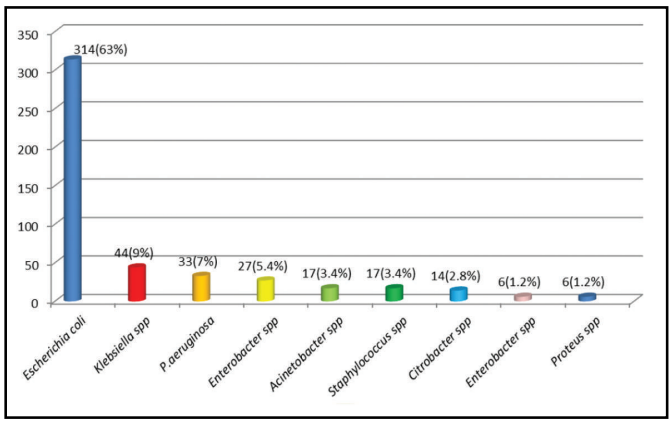

Figure 1: Number and percentages of different organisms isolated (n=500)

Table 1: Antimicrobial susceptibility pattern of *Escherichia coli* isolates (n=314)

Antibiotics	Sensitivity
Amikacin	253(81%)
Ampicillin	2(0.6%)
Amoxicillin-clavulanic acid	5(1.5%)
Ciprofloxacin	92(29%)
Ceftriaxone	118(38%)
Gentamicin	163(52%)
Co-trimoxazole	65(21%)
Nitrofurantoin	274(87%)
Piperacillin-tazobactam	287(91%)
Imipenem	301(96%)

Table 2: Antimicrobial susceptibility pattern of Enterobacteriaceae other than *E. coli* (n=70)

Antibiotics	*Klebsiella* spp. (n=44)	*Proteus* spp. (n=6)	*Enterobacter* spp. (n=6)	*Citrobacter* spp. (n=14)
Amikacin	31(70%)	4(67%)	4(67%)	5(36%)
Amox-clavulanic acid	17(39%)	2(33%)	1(17%)	2(12%)
Ciprofloxacin	16(36%)	4(67%)	2(33%)	3(21%)
Ceftriaxone	18(38%)	5(83%)	2(33%)	2(12%)
Piperacillin-sulbactam	25(57%)	5(83%)	4(67%)	2(12%)
Gentamicin	16(36%)	2(33%)	2(33%)	3(21%)
Co-trimoxazole	10(23%)	1(17%)	1(17%)	2(14%)
Nitrofurantoin	17(39%)	1(17%)	1(17%)	5(36%)
Piperacillin-tazobactam	31(71%)	6(100%)	4(67%)	5(36%)
Imipenem	38(86%)	6(100%)	6(100%)	10(71%)

Table 3: Antimicrobial susceptibility pattern of *Acinetobacter* spp and *P. aeruginosa*

Antibiotics	*Acinetobacter* spp (n=17) Sensitivity	*Pseudomona aeruginosa* (n=33) Sensitivity
Amikacin	4(24%)	19(58%)
Amoxicillin-clavulanic acid	4(24%)	
Ciprofloxacin	5(29%)	9(27%)
Ceftazidime	11(65%)	31(94%)
Ceftriaxone	11(65%)	
Piperacillin-sulbactam	12(70%)	24(72%)
Gentamicin	3(18%)	9(27%)
Co-trimoxazole	3(18%)	
Nitrofurantoin	2(12%)	
Piperacillin-tazobactam	16(94%)	25(76%)
Imipenem	12(70%)	27(86%)
Sulbactam-cefoperazone	16(94%)	26(78%)

Table 4: Antimicrobial susceptibility pattern of *Staphylococcus* spp and *Enterococcus* spp

Antibiotics	*Staphylococcus* spp (n=17) Sensitivity	*Enterococcus* spp (n=27) Sensitivity
Amikacin	11(65%)	2(0.7%)
Ampicillin	2(12%)	18(67%)
Amoxicillin-clavulanic acid	7(41%)	18(67%)
Ciprofloxacin	6(35%)	5(19%)
Gentamicin	9(53%)	
Co-trimoxazole	5(29%)	
Nitrofurantoin	7(41%)	23(85%)
Vancomycin	17(100%)	27(100%)
Teicoplanin	17(100%)	27(100%)
Linezolid	17(100%)	27(100%)
Doxycycline	6(35%)	6(22%)

Discussion

Antibiotic resistance is a serious public health threat. The antibiotic-resistant organisms can quickly spread and threaten communities with new strains which are more difficult and expensive to treat. Treatment failures may arise due to the resistance offered by pathogens against effective broad spectrum antibiotics. In a study conducted at Khyber Teaching Hospital, Peshawar in 2009, out of total of 342 culture positive samples, *E. coli* was isolated from only 33.9% cases,[9] while in our study *E. coli* was isolated from 63% of the total culture positive cases reflecting a much higher isolation of this pathogen in our setup.

Comparing the spectrum of our urinary pathogens with two studies carried out in India and Nigeria in 2009 it was found that *E. coli* was isolated from only 36% and 25% of the total culture positive samples from the two centres respectively, compared to 63% in our case.[10, 11] On the contrary, *K. pneumoniae* was isolated from much higher number of patients (22% and 17%) in their setup as compared to only 7% in our study. Our results were, however, in concordance with the study carried

out in Gaza, Palestine where *E. coli* accounted for 52% of the total culture positive isolates.[12]

Nitrofurantoin was found to be the most effective urinary antibiotic against *E. coli* and *Enterococcus* spp, in our study. Similar results were reported in 2004 from our centre, which revealed that 88% of *Enterococcus* spp isolated during the study period were susceptible to nitrofurantoin.[13] Nitrofurantoin has been considered as an option for empirical therapy by many authors in Latin America and United States, since the multiple mechanism of action of this antibiotic seem to have enabled it to retain its activity against urinary pathogens especially *E. coli.*[14-16] However, nitrofurantoin is only recommended for uncomplicated cystitis for antimicrobial prophylaxis of recurrent UTI.[17]

Although cotrimoxazole is also empirically recommended as one of the antimicrobial to treat uncomplicated cases of UTI,[18] our results revealed higher percentage of *E. coli* isolates resistant to this compound. Similar results were reported from studies carried out in Palestine and Brazil, where 64% and 58% of *E. coli* isolates respectively from urine culture were resistant to cotrimoxazole.[12, 19]

As regards the susceptibility of different urinary pathogens to ciprofloxacin, only 29% of our *E. coli* isolates and 35% of gram negative rods other than *E. coli* were susceptible. The results were comparable with the similar study done in Peshawar in 2005/ 2006 where 28% of the total *E. coli* isolates was susceptible to this compound.[9] It seems that our isolates have higher resistance to ciprofloxacin as compared to the studies reported from Palestine, Canada, USA and Turkey.[12, 20-22] The probable reason for higher resistance of our urinary pathogens against ciprofloxacin is widespread usage and over the counter easy availability of this drug.

The antimicrobial susceptibility profile of our *P. aeruginosa* isolates revealed that a high proportion of the isolates was susceptible to ceftazidime followed by imipenem 86%, sulbactam-cefoperazone 78% and piperacillin-tazobactam 76%. Only 27% of *P. aeruginosa* isolates were susceptible to ciprofloxacin. Similar results have been reported by a Polish study.[23]

In our study as majority of isolates were *E.coli*, the susceptibility result of these isolates definitely has statistical significance whereas remaining (37%) isolates were of diverse genera and as such the antimicrobial susceptibilities of these isolates should be interpreted with caution.

Clinical information was not available with the majority of the specimens received. Thus the patients were not stratified as symptomatic or otherwise; lack of clinical information was the weak link of our study. The availability of basic clinical information along with specimen helps the microbiologists in improving the interpretation of the culture results. Variety of factors including misuse of antibiotics by non skilled practitioners, health care professionals and general public, might have contributed to such higher rates of resistance to commonly used urinary antibiotics in our setup. Adequate surveillance based upon the information arising from routine antimicrobial susceptibility testing is therefore needed to find out our local pattern of resistance. Thus surveillance studies are recommended in Pakistan to monitor antibiotic susceptibility pattern in this region to help clinicians while using empirical treatment for infections. It would be fruitful for the clinical microbiological laboratories in different centres to publish their urinary antibiogram annually to observe early trends of emerging resistance and to counteract accordingly.

Conclusion

The antimicrobial susceptibility pattern of *E. coli* isolates revealed that majority of isolates were susceptible to imipenem and piperacillin-tazobactam, while susceptibilities to ampicillin, cotrimoxazole and ciprofloxacin (still considered as treatment of choice for UTIs) were low. Strict antibiotic policy should be adopted in hospitals to counter the impact of increased resistance in bacteria and to take necessary steps for preventing the development/reducing this resistance. Regular surveillance studies are recommended for appropriate guidance of the clinicians.

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ORIGINAL ARTICLE

Risk Factors for Acquiring MDR Pathogen in a Tertiary Care Hospital

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Abstract

Objective
To find out common risk factors in patients from whose samples Multi-Drug Resistant pathogens were isolated.

Study Design
Prospective observational study.

Setting
Microbiology department of Liaquat National Hospital Karachi, a 750-bedded tertiary care Hospital.

Material and Method
All multi-drug resistant pathogens (resistant to representative antibiotics of atleast three different classes of antimicrobial agents including carbapenems) isolated from samples like blood, bronchial wash, sputum, pus, etc, received from different units of hospital at Microbiology laboratory Liaquat National Hospital from December 2006 through February 2007 were included in this study. Patient information was collected from their personal file and through concerned treating physicians.

Results and Conclusion
A total of 228 MDR pathogens were isolated from different samples in 3 months, these included: *Acinetobacter* spp 184 (81%) and *Pseudomonas aeruginosa* 44 (19%). Majority were from lower respiratory tract specimen, followed by blood. Most (86%) patients were in intensive care unit or high dependency unit. Mechanical ventilation was predominant finding in patients with *Acinetobacter* spp while surgical procedures were more frequently associated with *Pseudomonas aeruginosa*. Only 3% of *Acinetobacter* spp and 7 % of *Pseudomonas aeruginosa* were isolated during first 48 hours of hospital stay.

Key words
Acinetobacter species, MDR Pathogen, *Pseudomonas aeruginosa,* Risk Factors, Ventilator.

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Introduction

Treating infections caused by Multi-drug resistant (MDR) pathogens is one of the greatest challenges in clinical practice in this era. Definitions for "Multi-drug resistant" or "Pan resistant" vary widely in the published literature.[1,2] At our institution, we define multidrug-resistant pathogen as an isolate resistant to representative antibiotics of atleast three different classes of antimicrobial agents including carbapenems. Most isolates that meet our definition are completely resistant to commonly prescribed antibiotics or are susceptible to only the aminoglycoside class of agents and colistin. Such MDR *Pseudomonas aeruginosa* and *Acinetobacter* spp which are resistant to carbapenems along with all other major groups of antibiotics are an emerging problem in most Intensive Care Units, with almost no antibiotic left for therapy. Empirical therapy with broad spectrum antibiotics like carbapenems and other combinations can also fail in these situations. Identifying patients at higher risk of developing infections with MDR pathogens can help guide clinicians in their choice of empiric antibiotics as well as implementing infection control practices.

Material and Methods

A study was carried out at Microbiology Department, Liaquat National Hospital, from 1 December 2006 to 28 February 2007. Bacterial isolates from the clinical samples showing resistance to three or more classes of antibiotics, including carbapenems, were labeled MDR pathogen and included in the study.

All samples received at the laboratory were processed as per standard microbiological protocols and API 20E strips (BioMerieux, France) were used for gram negative rods.[3] Bronchial washings were processed by quantitative methods, and bacteria present in 10^3 CFU/mL or more were processed. Antibiotic sensitivity testing was done by disc diffusion method (Kirby-Bauer) using CLSI standards.[4]

Sources of samples were recorded and patient information was gathered from personal files through ward visits. At the end of the study period, all the data were avaluated.

Results

A total of 228 MDR Gram negative rods were isolated, identified as either *Acinetobacter* species 184 (81%) or *Pseudomonas aeruginosa* 44 (19%). Of all isolates, 3% of *Acinetobacter* spp and 7 % of *Pseudomonas aeruginosa* were isolated within 48 hours of admission.

Majority of these isolates were from the lower respiratory tract samples; *Acinetobacter* spp (67%) and *Pseudomonas aeruginosa* (62%). The second frequent isolates were from blood; *Acinetobacter* spp (19%) and *Pseudomonas aeruginosa* (14%) (Table 1).

Table 1: Distribution according to samles

Sample	% *Acientobacter* spp	% *Pseudomonas aeruginosa*
Lower Respriatory tarct	62	67
(a) Tracheal Secretion	53	55
(b) Sputum	9	12
Blood	19	14
Pus	11	7
Urine	1	2
Others	7	10

Duration of hospitalization was more than 7 days in 67% patients. Among them 196 (86%) patients were either in ICU or HDU (Table 2)**.**

Mechanical ventilation was a predominant finding in patients who had MDR *Acinetobacter* spp (73%) as compared to MDR *Pseudomonas aeruginosa* (42%). Surgical procedures on the other hand were performed in 76% of patients with MDR *Pseudomonas aeruginosa* as compared to 31% among those who acquired MDR *Acinetobacter* spp.

Previous antibiotic exposure was assessed in 110 patients. Thirty percent were on Carbapenems, 20% on Beta-lactam /Beta-lactam combinations and only 5% on Quinolones.

Discussion

In this study we analyzed some of the risk factors among patients from whom MDR pathogens were isolated. Majority of these MDR pathogens were nosocomially acquired, as only 3% and 7% of isolates of *Acinetobacter* spp and *Pseudomonas aeruginosa* respectively were isolated during first 48 hours of admission. There is a chance that a patient could be harbouring MDR pathogen on admission, unless on-admission screening is done, and patient is placed in contact isolation to prevent spread to other patients.

Factors like stay in ICU/HDU, duration of stay and sample source were similar for both pathogens, however, important finding was that MDR *Acinetobacter* spp were more frequently isolated from patients on ventilator, and more so in patients on broad-spectrum antibiotics. On the other hand, MDR *Pseudomonas aeruginosa* was more frequently isolated in post operative infections or in patients who under went some surgical procedure.

Pseudomonas aeruginosa has been documented as a major cause of Ventilator associated Pneumonia (VAP); both exogenous and endogenous sources have been identified.[5] In a study of epidemiological risk factors for *Pseudomonas aeruginosa*, Thuong *et al* found association with prior antibiotic use (42%), ICU stay (71%), and assessed contribution of low level of preventive measure as one of the important factors.[6,7] Due to its minimal nutritional requirements it can grow in diverse conditions and has predilection for moist environment and

Table 2: Risk factors associated with two MDR pathogens

Organisms	ICU	HDU	ICU/HDU	Wards	<2 Days	>7 Days	Surgery	Mechanical ventilation	Total
Acinetobacter **species**	130 (71%)	28 (15%)	158 (86%)	26 (14%)	6 (3%)	127 (69%)	57 (31%)	134 (73%)	184 (81%)
Pseudomonas aeruginosa	20 (45%)	18 (41%)	38 (86%)	6 (14%)	3 (7%)	28 (63%)	33 (76%)	18 (42%)	44 (19%)
Total	150 (66%)	46 (20%)	196 (86%)	32 (14%)	9 (4%)	155 (67%)	90 (39%)	152 (66%)	228

Note: Difference in two species were compared according to location, days post admission, and correlation with surgical procedures and mechanical ventilation.

disruption of normal skin or insertion of medical devices.[8] Previous antibiotic exposure and moist environment for transmission of this organism has repeatedly been stressed.[9]

Aqueous solutions used in medical care e.g., disinfectants, soaps, irrigation fluids, eye drops, and dialysis fluids, can become contaminated with *Pseudomonas aeruginosa*. It is also frequently found in the aerators and traps of sinks, in respiratory therapy equipment. It may contaminate bronchoscopes and lead to outbreaks of infection. Long or artificial fingernails may also harbor *Pseudomonas aeruginosa* and may be associated with outbreaks.[2] However, person to person transmission has been assessed as less significant; 18% in a recent survey.[10] Contribution of previous quinolone therapy has been considered a risk factor which was not the case in our study. [11]

Risk factors for acquiring *Acinetobacter* spp are somehow similar. As the organism can survive in dry and moist conditions for a prolonged period, it is not surprising that even dry parts of the hospital environment may be potential reservoirs of pathogens. *Acinetobacter* spp (predominantly *A. baumannii*) causes infection almost exclusively in the hospital setting and particularly in patients in ICUs.[2,12] It causes VAP and in outbreak situations can be very difficult to control in units with large numbers of patients undergoing ventilation.[13] None of the studies have quantified the attributable fraction due to patient-to-patient transmission versus antibiotic selective pressure for acquisition of *A. baumannii*.[14] Role of environment, both moist and dry, including healthcare workers contribute in spread of *Acinetobacter* spp, it has capacity to persist on environmental surfaces for long duration as long as 25 days.[15]

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In Vitro Efficacy of Nitrofurantoin Against Various Urinary Isolates

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Abstract

Objective
To evaluate the *in vitro* activity of nitrofurantoin against various isolates yielded from urine specimens.

Place and duration
Department of Microbiology, Armed Forces Institute of Pathology Rawalpindi, from January 2009 through January 2010.

Materials and methods
A total of 919 urinary isolates (*Escherichia coli* 690, *Klebsiella pneumoniae* 62, *Klebsiella oxytoca* 26, *Enterococcus faecalis* 54, *Enterococcus faecium* 18, *Enterobacter agglomerans* 16, *Enterobacter cloacae* 4, *Acinetobacter baumannii* 20, *Citrobacter* spp 15, *Serretia marcesens* 14) were cultured from urine specimens during the study period. All the isolates were tested against nitrofurantoin along with other routine antibiotics used for urinary pathogens.

Results
Out of total 919 urinary isolates, percentages of sensitivities of nitrofurantoin against *Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Enterococcus faecalis, Enterococcus faecium, Enterobacter agglomerans, Enterobacter cloacae, Acinetobacter baumannii, Citrobacter* spp, *Serretia marcesens* were 89.4, 32.3, 15.4, 81.5, 39.0, 43.8, 50, 25, 26.7 and 28.6% respectively.

Conclusion
Nitrofurantoin is very effective against urinary isolates *in-vitro* and can be used empirically for uncomplicated urinary tract infections.

Key words
Nitrofurantoin, Urine Culture, Urinary Tract Infections.

Introduction
Urinary tract infections (UTIs) are the second most common infections in the community after respiratory tract infections.[1] About 8 to 10 million patients visit clinicians for UTIs each year in the united states.[2,3] Gram negative organisms specially *E. coli* are the leading cause of UTIs and bacteremia in the elderly, causing significant mortality and morbidity.[4] It is estimated that about 10 to 20% of women will have atleast one UTI in their life time.[5] Unfortunately, exact incidence of UTIs caused by various organisms in Pakistan is not well known.

Ampicillin and sulfamethoxazole-trimethoprim were frequently used for the treatment of uncomplicated UTIs in the recent past.[6] American Infectious Disease Society in 1990s documented use of sulfamethoxazole-trimethoprim as first line drug for treating acute cystitis in women[7]. Due to its overuse and misuse, increased resistance has been reported worldwide.[6,8-10] Second option is a fluoroquinolone in sulfamethoxazole- trimethoprim resistant areas. However, many clinicians still believe that excessive use of fluoroquinolone for cystitis is inappropriate possibly due to its cross-resistance.[11] In this situation, there is a need to rethink about first and second line treatment options in uncomplicated UTIs. Nitrofurantoin is effective against both gram negative and gram positive pathogens. It has less side effects, rare resistant mutants and there is no cross reactivity with other antimicrobial agents. It has given good results even against multi-drug resistant urinary pathogens. This study was planned to determine the efficacy of nitrofurantoin against various urinary isolates cultured in a reference laboratory so as to weigh its empirical recommendation as first line agent in uncomplicated UTIs.

Materials and methods
This laboratory based descriptive study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi from January 2009 through January 2010. All the urine specimens (centrifuged) having more than 4-5 pus cells/HPF were included in the study. No discrimination was made on the basis of age and gender. Specimens yielding growth of mixed organisms were excluded from the study. A total of 919 isolates from urine specimens were included during one year study period. In the protocol followed, all the urine samples were inoculated on CLED (Oxoid, UK) medium and incubated at 37ºC for 24-48 hours. Colonies were subjected to Gram stain and rapid tests. Confirmation and species

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differentiation was done with traditional biochemical tests by using API system (Biomerieux, France). Antimicrobial susceptibility testing was carried out on Muller Hinton Agar (Oxoid, UK) with nitrofurantoin 300µg (Oxoid, UK), ampicillin 10µg (Oxoid, UK), doxycycline 30µg (Oxoid, UK), gentamicin 10µg (SPAN Diagnostic, France), ceftazidime 30µg (Oxoid, UK), tazobactem/piperacillin 10/100µg (Oxoid, UK), sulbactam/cefoperazone 105µg (Oxoid, UK), trimethoprim/sulfamethoxazole 1.25/23.75µg (Oxoid, UK), vancomycin 30µg (Oxoid, UK), and imipenem 10µg (Oxoid, UK), by using modified Kirby-Bauer disk diffusion method.[12] *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 (ß-lactamase negative) and *Pseudomonas aeruginosa* ATCC 25853 were used as control strains.

Results

Out of total 919 positive specimens, 588 (64%) were from female patients and 331 (36%) from male patients. Age of the patients ranged 4 to 81 years (mean age ± SD was 31 ± 11.3 years) with maximum number of patients in the third decade. *E. coli* was the predominant isolate followed by *K. pneumoniae*

Table 1: Frequency of different organisms isolated (n = 919)

Organism	Total Number
E. coli	690
K. pneumoniae	62
K. oxytoca	26
E. faecalis	54
E. faecium	18
Enterobacter agglomerans	16
Enterobacter cloacae	4
A. baumannii	20
Citrobacter spp	15
Serratia marcesens	14

(Table 1).

Nitrofurantoin was effective in 89.4% in *E. coli* isolates followed by *E. faecalis* (81.5%) and *Enterobacter cloaca* (50%). Overall 714 (78%) of the isolates were sensitive to nitrofurantoin (Table 2). *Klebsiella* isolates were most resistant to nitrofurantoin followed by *A. baumannii*.

Discussion

In uncomplicated UTIs, the drug of choice should be safe, effective and appropriate. In a survey done at emergency department, 100 patients suspected of UTI were prescribed fluoroquinolones and out of them 81% were given inappropriate treatment.[13] Nitrofurantoin has emerged as "the return of an old friend in the wake of growing resistance".[7] It hardly disturbs normal intestinal flora and is well tolerated. However, local

Table 2: ***In vitro*** **susceptibility of urinary tract isolates to nitrofurantoin (n=919)**

S.#	Isolate	n	Nitro -resistant	Nitro -sensitive
1	*E. coli*	690	73 (10.6%)	617 (89.4%)
2	*K. pneumoniae*	62	42 (67.7%)	20 (32.3%)
3	*K. oxytoca*	26	22 (85%)	4 (15.4%)
4	*E. faecalis*	54	10 (19%)	44 (81.5%)
5	*E. faecium*	18	11(61.1%)	7(39%)
6	*Enterobacter agglomerans*	16	9 (56.3%)	7 (43.8%)
7	*Enterobacter cloacae*	4	2 (50%)	2 (50%)
8	*A. baumannii*	20	15 (75%)	5 (25%)
9	*Citrobacter* spp	15	11(73.3%)	4 (26.7%)
10	*Serratia marcesens*	14	10 (71.4%)	4 (28.6%)
	Total	919	205 (22%)	714 (78%)

antibiotic policy and sensitivity pattern should be kept in mind. Various studies have been done worldwide to evaluate the performance of nitrofurantoin.[14-16] Resistance to nitrofurantoin still remains low, 3 to 11%.[17] It has been used as first line drug in some of the countries like Norway, Sweden while its use is still controversial in Germany potentially because of its serious side effects (polyneuropathy, interstitial pneumonia).[17] However, these side effects are very rare and occur only after prolonged therapy.

In Pakistan, very few studies have reported on the efficacy of nitrofurantoin. In one study done to evaluate the activity of nitrofurantoin against enterococcus isolates, out of total 144 isolates, 127 (88%) were sensitive to nitrofurantoin.[1] In our study, 71% isolates were sensitive to nitrofurantoin. In an other study, more than 80% of the UTIs were caused by *E.coli*[18]. In our study, *E. coli* was the predominant isolate with 89.4% sensitivity to nitrofurantoin. Our results are comparable to another Canadian study in which out of total 2000 urinary tract isolates, 84.1% isolates were *E. coli* and mean rate of resistance to nitrofurantoin was 0.1%.[19]

In our study, *Klebsiella oxytoca* was the least sensitive isolate against nitrofurantoin preceded by *A. baumannii* and *Klebsiella pneumoniae*. In the previously mentioned Canadian study, *Proteus mirabilis* and *Klebsiella pneumoniae* showed greatest resistance against nitrofurantoin.[19] We did not check the susceptibility of nitrofurantoin against *Proteus* spp because nitrofurantoin activity is reduced in alkaline pH. In our study, overall sensitivity of nitrofurantoin against urinary isolates was 78%. In a study done by James *et al*, sensitivity of nitrofurantoin was 95.6%.[7] Lesser sensitivity rates in our

study as compared to above mentioned study may due to difference in the patient population. In our study, urine specimens both from outpatients and hospitalized patients were included; hospitalized patients have more complicated UTIs and isolates are more resistant to antibiotics than outpatients.

As it is a laboratory based study, more studies should be done on outpatients and inpatients separately to know the exact sensitivity of nitrofurantoin against urinary isolates. The main problem with this drug is its availability in the market.

Conclusion

Our results suggest that most of the uropathogens are susceptible to nitrofurantoin *in vitro*. It is cheap, effective and can be used for long periods in recurrent and chronic UTIs. It can be used empirically in uncomplicated UTIs thus sparing fluoroquinolones.

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ORIGINAL ARTICLE

Assessment of Febrile Children Younger than 5 Years of Age at POF Hospital, Wah - A Clinical Audit

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Abstract

Objective
To audit the clinical practices regarding assessment of febrile children younger than 5 years of age at POF Hospital Wah Cantt.

Methodology
This clinical audit was performed at department of pediatrics POF Hospital, Wah during June 2010. Clinical charts of all the children younger than 5 years of age admitted with presenting complaint of fever were analyzed using specially designed audit proforma. National Institute for Health and Clinical Excellence (NICE) Guidelines for assessment and initial management of feverish illness in children younger than 5 years of age was used as reference standard.

Results
A total of 134 clinical charts were included in the audit. Gender distribution was 47% female and 53% male. Age ranged between 2 to 60 months (mean age 24.6 months). Children 6 to 18 months of age formed the major proportion (46%). Diarrhoea was the most common associated complaint (32.8%). Convulsions were associated in 5.9% cases. Duration of fever before presentation was 1 day(23.5%), 2-3 days(37.8%) and more than 3 days(38.7%). Antipyretics were used in 90.6% and antibiotics were used in 57.7% cases before presentation. Body temperature was documented in 53.8% cases.

Clinical assessment was completely documented according to reference standard in 45.4% cases. The essential parameters of heart rate, respiratory rate and capillary refill time were recorded in 79.8%, 55.5% and 25.8% cases respectively. All three parameters were documented in 26% cases.

Conclusion
The audit demonstrated discrepancies between existing practices and the standard guidelines. There is a dire need of implementing the standard guidelines in true spirit.

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Key words
Fever, Medical Audit, NICE Guideline, Traffic Light System.

Introduction
Fever is defined as controlled rise in body temperature above normal values for that age[1]. Fever is one of the most common presenting complaints in pediatric patients of all age groups and especially in children under five years[2,3]. As a result assessment of febrile children of different age groups is a challenge faced by physicians working in outpatients as well as in Accident & Emergency department. In pediatric age group fever is most commonly associated with infectious diseases, bacterial or viral, some of which can be life threatening[4]. Quite frequently fever in a child is not the result of a serious illness but still is a cause of parental concern and anxiety especially in young children[5]. Only a thorough evidence based assessment can enable a physician to decide the optimum care for a particular child on one hand and allay the parental anxiety on the other. Such assessment also minimizes the risk of missing the diagnosis of serious illnesses. This fact is even more important in developing countries like Pakistan where infections are the major cause of childhood mortality and morbidity. Keeping in view all these aspects evidence based guidelines have been developed by different international health authorities[6,7,8]. Such guidelines ensure standard case management of febrile children at all levels of care and thus minimize the gap in patient care available to different segments of society. This in turn will help in reducing childhood mortality and morbidity.

Regular clinical audits should follow the implementation of such evidence based guidelines to monitor and ensure compliance to best practices. Present clinical audit was under taken to evaluate the assessment of febrile children at Pediatrics Department POF Hospital, Wah Medical College using NICE guidelines for feverish illness in children assessment and initial management in children younger than 5 years as reference standard[8].

Methodology
POF Hospital is a tertiary care teaching hospital of Wah Medical College. Pediatrics department is an eighty bed facility with

pediatric emergency, ITC and NICU. As a departmental policy NICE guidelines for assessment and initial management of feverish children younger than five years of age are followed as standard practice[8].

This was a clinical chart audit conducted at pediatrics department POF Hospital, Wah Cantt. The audit period was 1st June to 30th June 2010. The clinical audit was approved by Ethics Committee POF Hospital.

It was aimed to review consecutive case charts of children age one month to 5 years who presented to pediatric emergency room with presenting complaint of fever. Data was extracted from the clinical charts on an audit proforma especially designed for this study.

These charts were analyzed to specifically evaluate:

1. Percentage of children assessed for the presence or absence of signs of serious illness according to 'traffic light system' for identifying risk of severe illness as per NICE guidelines[8]. Traffic light system is a clinical tool used to predict the likelihood of serious illness in febrile children. Children with all the signs and symptoms in green column and no clinical feature from red or amber column are at low risk. Children with one or more features from amber column are at intermediate risk and those with any feature from red column are at high risk of serious illness.
2. Percentage of children whose temperature, heart rate, respiratory rate and capillary refill time have been measured and recorded as part of the routine assessment as recommended in reference standard guidelines.

The data was collected by an audit team of pre-trained health personnel including doctors and senior nurses. The data was collected analyzed and charted using Microsoft Excel for Windows. Percentage compliance to the reference standard was calculated.

Results

During June 2010, a total of 187 children presented to emergency room of pediatrics department with complaint of fever. Among these, 42 children were out of the age range selected for the audit. In the rest of records, 11 charts were incomplete and not included in the audit. As such, 134 charts were reviewed for the audit purposes as shown in figure 1.

Age range was 2 months to 60 months (mean=24.6 months+ 17.04 months). Gender distribution analysis revealed that 64 (47.7%) cases were female and 70(52.2%) male. Children with age 6 months to 18 months formed the largest proportion in this audit (46%); children < 6 months were 23% and >18 months to 5 years were 31% of the total cases.

Associated presenting complaints were recorded (table 1).

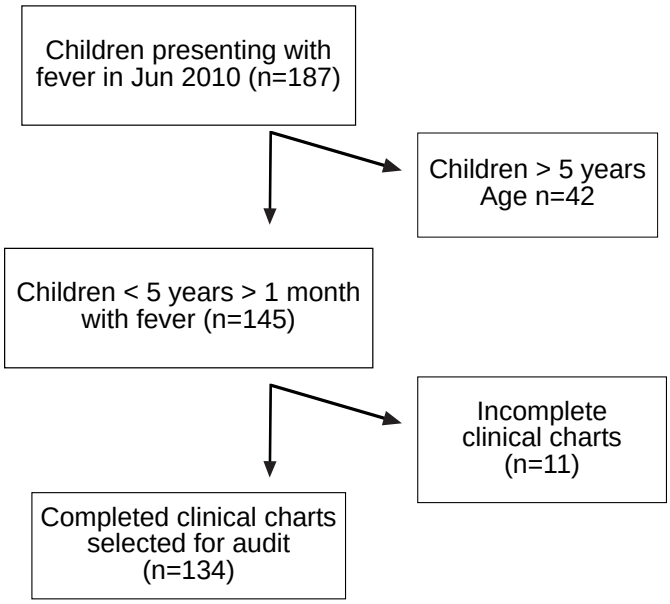

Figure 1: Flow of clinical charts through the audit (n=187)

Table 1: Relative frequency of associated complaints in patients (n= 134)

Associated complaints	n	%age of total
Loose motions	44	2.8
Vomiting	23	17.2
Cough or breathing difficulty	17	12.7
Rash	10	7.5
Poor feeding	9	5.9
Convulsions	9	5.9
Urinary complaints	5	3.7
Others	7	5.2
No associated complaints	10	7.5

Duration of fever was also noted. It was found that 23.5% patients reported on first day of illness, 37.8% reported between second and third day and 38.7% presented with history of fever more than 3 days.

90.6% children received antipyretics before coming to hospital, 57.7% received antibiotic and in 22% cases tepid water sponging was attempted to reduce fever.

Among the 9 (5.9%) children with convulsion as associated chief complaint, 48% were having first convulsion in life while 52% had a previous history of convulsion with or without fever.

When physical assessment as documented on the clinical charts

was reviewed, it was found that in 53.8% cases fever was measured and documented in ºF while in 46.2% cases fever was documented as high grade or low grade without measurement. Instruments used to measure temperature were mercury thermometer placed in axilla in 45.5% cases, forehead chemical strip in 23% cases and infrared forehead scanner in 31.5% cases.

Step by step review of charts was done for assessment according to traffic light system for identifying risk of serious illness as given in reference guidelines (table 2). Firstly assessment of red (high risk) features was reviewed and it was found that in 89 (66.4%) cases, all the features were assessed for presence or absence and documented on the charts. Similar audit for amber (intermediate risk) and green (low risk) features revealed that in 63 (47.0%) and 78 (58.2%) cases respectively the assessment was done and documented completely. On the aggregate 61 (45.4%) cases showed complete assessment according to traffic light system for identifying risk of severe illness.

Assessment of essential features like heart rate, respiratory rate and capillary refill time showed that heart rate was documented in 107 (79.8%) charts. Respiratory rate was documented as tachypnoea without giving actual rate in 23% cases while in 21.5% cases respiratory rate was not documented at all. Capillary refill time was checked in 25.8% cases, it was not documented

Table 2: Traffic light system assessing the risk of serious illness in feverish children under 5 years.[8]

	Green (Low risk)	Amber (Intermediate risk)	Red (High risk)
Color	• Normal color of skin, lips and tongue	• Pallor reported by parent or carer	• Pale, mottled, ashen or blue
Activity	• Responds normally to social cues • Is content or smiles • Stays awake or wakes quick • Strong normal cry or not crying	• Doesn't respond normally to social cues • Wakes only with prolonged stimulation • Decreased activity • No smile	• No response to social overtures • Appears ill to healthcare professional • Unarousable or does not stay awake if aroused • Weak, high pitched or continuous cry
Respiration	• Normal	• Nasal flaring • Tachypnoea: respiratory rate >50 breaths/min (age 6-12 months) Or >40 breaths/min (age 12 months) • Oxygen saturation < 95% in air • Crackles on auscultation	• Grunting • Tachyponea; respiratory rate 60 breaths/min (at any age) • Moderate to severe chest indrawing
Hydration	• Normal skin and eyes • Moist mucous membranes	• Dry mucous members • Poor feeding in infants • Capillary refill time > 3 seconds • Reduced urine output	• Reduced skin turgor
Other	• No amber or red features	• Fever for > 5 days • Swelling of a limb or joint • Not weight bearing or not using an extremity • A new lump >2 cm	• Temperature > 38^0C (age 0-3 months): >39^0C (age 3-6 months) • Non-blanching rash • Bulging fontanelle • Neck stiffness • Status epilepticus • Focal neurological signs • Focal seizures • Bile stained vomiting

in rest of the cases. A complete assessment of all three essential features was done in 26% cases.

Discussion

The audit demonstrated largest proportion of children included were less than 2 years of age. Other studies have also highlighted the fact that young febrile children are more likely to present and get admitted with febrile illnesses[9]. It has been seen that duration of fever is not related to severity of illness[10,11]. However, it was found in the present audit that maximum proportion of children presented later than second day of illness. The most common associated problem with fever in the present study was loose motions. This may be attributable to the fact that study was conducted during summer time when diarrhoea is most prevalent.

This clinical audit revealed many findings reflecting the gaps in assessment of febrile children at pediatrics ward POF hospital. Recording of temperature and documenting the finding is the first and very important step in evaluation. In the present study, omission of documenting temperature was seen quite frequently. Use of the instruments like forehead chemical strip or infrared forehead scanner was observed in large proportion of patients. Accuracy and reliability of these instruments is not confirmed by past studies so their use remains at odds with the evidence based guidelines[12,13].

Alarmingly less than half of the charts included in the audit showed complete assessment based on traffic light system of reference guidelines. Analysis showed the tendency of assessing red column features more frequently whereas assessment of amber and green columns is also important for risk assessment in a febrile child.

One of the major concerns from this audit was the fact that essential features like respiratory rate, heart rate and capillary refill time were not recorded in majority of cases. Tachypnoea has proven to be a reliable sign of pneumonia, a life threatening infection in children less than 5 years of age[14]. Thus recording of respiratory rate forms an essential part of assessment of a febrile child. However, in the present audit failure to document or exactly record respiratory rate was observed in many cases making the follow-up evaluation of sick child very difficult.

Clinical studies showing normal heart rates at different ages and its variation with body temperature do not give uniform information to be followed as guidelines [15]. Still measurement of heart rate is an important tool to assess the circulatory status of a child [16]. That is why this measurement is a part of evaluation of febrile child. However the compliance to this recommendation was not up to the mark in the present audit.

Capillary refill time test is also carried to for the assessment of circulatory status and prolonged capillary refill time has been found a reliable sign of shock[17, 18]. The present audit demonstrated low compliance to this practice.

Many studies advocate detailed clinical assessment as the sole way to diagnose cause of fever and risk assessment[19, 20]. Failure to do complete evidence based clinical assessment of febrile child leads to failure to detect high risk patients. This also leads to over investigating low risk or standard risk patients and administration of undesirable treatments [9,20]. This can be avoided only by following evidence based clinical assessment guidelines. The issues highlighted are the same across various clinical settings in different countries [20,21].

In response to the present audit, reinforcement of the standards was done. Group discussions, presentations and feedbacks were arranged for the doctors in the unit to increase the compliance to the standards and to correct the previous omissions. Guidelines especially the traffic light table were displayed clearly in outpatient as well as accident and emergency departments. Training of doctors was arranged for recording respiratory rate and performing capillary refill time test. The present audit is to be repeated on six monthly basis for continuing compliance.

Conclusion

The audit demonstrated discrepancies between existing practices and the standard guidelines. There is a dire need of implementing the standard guidelines in true spirit.

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Chickenpox in Taluka Khipro, District Sanghar Sindh

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Abstract

Chickenpox, a droplet infection caused by varicella zoster virus, is a highly infectious acute disease having vesicular rash accompanied by fever and malaise.

Objectives
The objectives of the study were to survey chickenpox disease in rural and poorly developed areas in order to record age, symptoms and seasonal trends in the rural setting.

Place of Study
Nine union councils of Taluka Khipro, District Sanghar, Sindh

Period of Study
January 2008 to December 2008.

Study Design
Cross sectional study

Material & methods
The information collected from 223 chickenpox cases reported from 9 union councils was recorded on a pre-tested designed questionnaire. Lady health workers and medical officers were trained and involved in the study.

Results
Highest number of cases reported from UC Kamil Hingoro (n=65) while lowest number from UC Dhilyar (n=7). Majority of the sufferers (67) belonged to 5 to 10 year's age group whereas the lowest incidence (8) was seen in age under 1 year. The leading symptoms were rash, fever and itching. There was no sex predilection for the disease. Most of the children suffering from chickenpox belonged to labourer and farmer families. The rural doctors were competent to diagnose clinically and common symptoms were identified by them.

Keywords
Chickenpox, Varicella Zoster Virus.

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Introduction
Chickenpox is a viral infection caused by Varicella Zoster virus (VZV). The virus belongs to alphaherpesvirinae subfamily and is a double stranded DNA virus. The disease is mainly a childhood exanthem. 90% children contract this infection before the age of 10 years[1]. In Pakistan, a study indicates that the seroprevalence of VZV increases with age[2]. The children of the mothers who are immune from chickenpox by natural infection or by getting vaccination of VZV are protected in their first year of life[3]. The disease presents with the symptoms which include headache, loss of appetite, weakness. Within 2 to 4 days itchy rash and vesicles appear all over the body and the face. The vesicles convert into blisters. These blisters become dry and their crusts fall without scars. There is a fatal condition called fetal varicella syndrome in newborn with multiple cutaneous scars, limb defects and ocular and brain problems[4]. There is renewed interest in epidemiology of VZV at an international level to develop an effective vaccine. The work on vaccine production was started in seventies and was used for the first time in hospitalized children in 1974. All the vaccinated children remained disease free[5].

Our objectives were to survey the prevalence of chickenpox disease in rural areas and record the age, symptoms and seasonal trends. The data would be used to evolve preventive strategies relevant to the rural setting.

Methodology
A survey of Chickenpox outbreak reported at Taluka District was conducted from January to December 2008. The authorities reported that Chickenpox cases were occurring and presenting in the health facilities in large number, therefore public health measures be suggested, hence the survey was designed with the help of Taluka Health Officer. This cross-sectional study was conducted jointly by Taluka Health Officer Khipro and the Department of Community Medical, Liaquat University of Medical & Health Sciences, Jamshoro. The union councils were selected from the official record. This list of the union councils was obtained and all the twelve unions of Taluka were included in the survey, to get the reasonable sample of the cases for the study. Patients of all ages reporting to the government health

facilities were included in the study. Those who were treated by private health care providers were excluded from the study. A pre-tested especially designed questionnaire was filled for every patient. Doctors in charge of the basic health units and lady health workers were trained in filling the questionnaire from the patients and quality was monitored by the Taluka medical officer. The questionnaire was also translated in to Sindhi and Urdu languages.

Results

Chickenpox cases were reported from 9 union councils. Highest number of cases 65 (29.2%) were reported from union council Kamil Hingoro while lowest cases 7 (3.1%) were from UC Dhilyar (Table 1). Male to female ratio was 1:1. Varicella infection was most prevalent in the age group 5 to 10 years and least prevalent under 1 year of age (Table 2).

Most of the patients belonged to working class and rural families (Table 3). 58.3% were not educated and 41.7% had some education (Table 4). The leading symptoms were rash and fever followed by itching (Table 5). Figure 1 shows rash presentation in different age groups.

The rural doctors were treating cases based upon symptoms and under guidelines given by EDO health; same were used for diagnosis of chickenpox. The most common months in which chickenpox cases were reported were May, June and July.

Discussion

This study identified that chickenpox affected both children and adults which was unusual presentation of the diseases. The disease was more common during May to July period which was different as literature reported February to April spring season[2].

Our study showed that disease had no preponderance for any sex. The disease was less prevalent in children below 5 years of age with lowest incidence below 1 year of age. The older

Table 1: Union Council wise cases of Chickenpox in Khipro (n=223)

S.No	Union Council	Population of Union Council	Chickenpox Cases (n)
1	Khipro	36765	32
2	Khori	34797	14
3	Qazi Faiz M. Rajar	32082	54
4	Kamil Hinogoro	36932	65
5	Hathungo	39944	21
6	Dhilyar	36990	7
7	Yamin Hingorjo	36840	0
8	Roonjho/Bhit Bhitai	32399	0
9	Khahi	38417	30
	Total	**288401**	**223**

Table 2: Age Wise distribution of cases (n=223)

Age	Numbe	%age
0-1 years	8	3.6
1-3 years	21	9.4
3-5 years	36	16.1
5-10 years	67	30.5
10 – 17 years	45	20.2
18 years & above	46	20.6
Total	**223**	**100 %**

Table 3: Distribution according to occupation of family head of patients

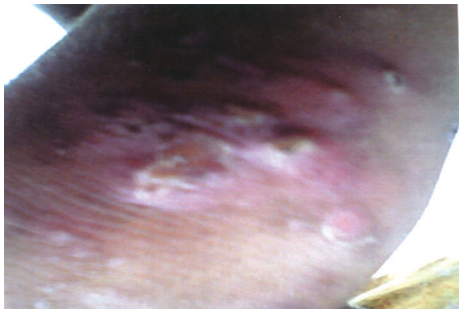
Occupation	Total	%age
Farmer	80	35.9
Labourer	73	32.7
Servicemen	30	13.5
Businessman	28	12.6
House wife	12	5.4
Total	**223**	**100 %**

Table 4: Distribution according to the education level of the family head of the patients

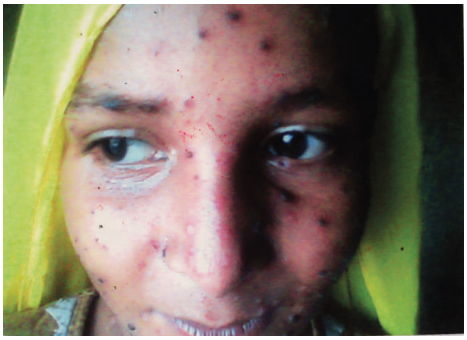
Education	Total	%age
Educated	93	41.7
Uneducated	130	58.3
Total	**223**	**100 %**

Table 5: Distribution according to symptoms

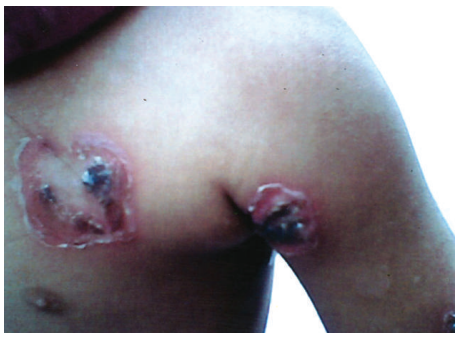
	Total	%age
Rash / Fever	190	85.2
Itch	30	13.5
Vomiting	2	0.9
Vertigo	1	0.5
Total	**223**	**100**



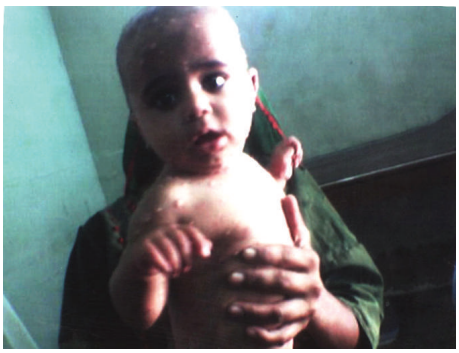
Rash of Chickenpox with abscess formation over arm



Rash over face of an adult female



Chickenpox crust on chest and arm of a child



Child showing early chickenpox rashes on body & face

Figure 1: Original photographs of patients taken at Taluka showing rashes (Permission for printing taken by the patients)

age between 5 – 15 years had higher incidence. This was due to over crowding vs greater chance of droplet infection and exposure at schools.

Disease was more prevalent in uneducated group due to lack of awareness and poor hygiene. The rash, fever and itch were main prodromal symptoms. Vomiting and vertigo were rarely seen in this study group.

The good point was that that rural health facility like Taluka Hospital and basic health unit successfully managed and treated this infection. Rural health system had trained doctor in common diseases through health management information system (HIMS) and diseases early warning system (DEWS), through this capacity building they were able to recognize diseases like chickenpox earlier.

In Taluka, skin specialist and child specialist were available for reconfirmation of the cases. The same skilled manpower was used for raising awareness among the locals. Policies were formulated at the local levels to deal with such outbreaks in future.

We searched for patterns of the chickenpox as reported in literature. These relied on clinical diagnosis rather than laboratory tests and chickenpox below the age of 10 years was a common finding[6-8]. Like our study, Bhalwar identified that the pattern for chicken pox infection was changing from childhood to adult population in the tropical countries[6]. There are multiple laboratory tests available for chickenpox diagnosis but still the clinical and epidemiological features are used for diagnosis[7]. Laboratory diagnosis otherwise may rarely be required as clinical signs are evident. The most rapid diagnosis can be done by examining fluid from the vesicles/rash under an electron microscope. There is still no specific treatment for chicken pox[8]. Our and published studies in India and EMRO region have common clinical findings but occurrence in late summer remains a dissimilarity.

Conclusion

This study identified outbreak of chickenpox in Khipro and least developed areas of Sindh, more pronounced during May to July period. More awareness and vigilance about chickenpox diagnosis, vaccination and treatment is needed in the backward areas.

Acknowledgment

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UPCOMING EVENTS

First International Biosafety Conference
November 18-19th 2011
Karachi, Pakistan

Pre-Conference Workshops
"Building Regional Biosafety and Global Ties"
"Biosafety Challenges in Resource Limited Countries"

Organizer: Pakistan Biological Safety Association

Epidemiology, Immunosuppression, Diagnosis, Treatment - Chlamydia & TORCH Infections
Kiev Ukraine
November 23, 2011

15th International Congress on Infectious Diseases
Bangkok
June 13-16, 2012

Instructions for Authors

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The Infectious Diseases Society of Pakistan sponsors the Infectious Disease Journal of Pakistan (IDJP). 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; with laboratory, clinical or epidemiological aspects.

Criteria for publication
All articles are peer reviewed by the IDSP panel of reviewers. Authors may also submit the names and contact information of two persons who potentially could serve as unbiased and expert reviewers for their manuscript, but IDSP reserves the right of final selection.

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Please submit the manuscript and all enclosures to: The Editor, Infectious Diseases Journal of Pakistan, Department of Pathology, Combined Military Hospital, Quetta, Pakistan. Electronic copy of the manuscript must also be sent to maahin1@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'*.

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Original Articles
Articles should report original work in the fields of microbiology and infectious diseases.

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This should list title of the article, full names of each author with highest academic degree(s), institutional addresses and email addresses of all authors. Corresponding author should also be indicated with his/her name, address, telephone, fax number and e-mail address. A short running title of not more than 40 characters (including letters and spaces) be placed at the foot end of the title page.

Abstract
Abstract should not exceed 200 words and must be structured in to separate sections. Avoid abbreviations or citing references in the abstract.

Key Words
Four to five standard key words must be provided.

Introduction
The section must clearly state the background and importance of the research along with objectives.

Materials and Methods
Mention the place and duration of study. design of study and details of interventions used must be clearly described. Provide details of subject selection (patients or experimental animals). Details must be sufficient to allow other workers to reproduce the results. Identify precisely all drugs and chemicals used, including generic name(s) and route(s) of administration.

Results
Present results in logical sequences in the text, tables and illustrations. Articles can have a maximum of five illustrations (in a combination of figures and tables) per article.

Discussion
Emphasize important aspects of the study and compare with other studies. Do not repeat the details from the results section. Discuss the implications of the findings and their limitations. 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.

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Authoritative and state of the art review articles on topical issues are also published, with a word limit of 2000. These should be comprehensive and fully referenced. Articles should contain Abstract, main mext divided into sections, Conclusions and References.

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Short clinical and laboratory observations are included as Brief Reports. The text should contain no more than 1000 words, 2 illustrations or tables and up to 10 references.

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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 and 2 illustrations or tables. The authorship should not exceed 4 persons.

Letter to the Editor
These may relate to material published in the IDJP, topic of

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Pertinent news and updates in infectious diseases from around the world (approximately 200 words).

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 in the order in which they are first cited in the text. Label 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.

Write all authors, complete title, journal name (standard abbreviation in italics), year, volume, 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. Table should be numbered consecutively in Arabic numerals. Tables and Figures legends should be self-explanatory with adequate headings and footnotes.

Illustrations
Illustrations should be numbered and given suitable legends. 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 Illustration
- Line, e.g. graphs: 600 dpi
  Halftone, e.g. photographs: 300 dpi

Coloured illustrations: 400dpi (images should be split CMYK)

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The research project must be reviewed by Ethical Committee of the institute. All clinical research articles/studies involving human subjects submitted to the IDJP must adhere to ethical guidelines of their institutions and have informed consent from their patients. All scientific research that uses animals in their study protocols must include a statement on the ethical treatment of animals during the study.

Financial support: Authors must disclose any financial support received during the course of the study.

Conflict of Interest: If there is any conflict of interest then this must be disclosed by the author(s) at the end of the article.

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Instructions updated - December 2010.
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