ORIGINAL ARTICLE

Demographics and clinical manifestations of patients with *Raoultella terrigena* infections: A Retrospective Single Center Study from Karachi, Pakistan

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ABSTRACT

Background: *Raoultella terrigena* (formerly *Klebsiella terrigena*) is an environmental gram-negative rod. It can cause infections in humans, especially in immunosuppressed patients and tends to be multi-drug resistant, limiting treatment options. There is lack of data on clinical presentation and outcomes of infections due to this organism. In this study; we describe the clinical features (presenting complaints, co-morbid diseases, complications, etc.), available treatment options, and outcomes (hospital/ICU stay, mortality) of patients with *R. terrigena* infections, seen retrospectively over six years.

Material and Methods: A cross-sectional study was conducted on all adult hospitalized patients with clinical specimens positive for *Raoultella terrigena* at a 700-bedded tertiary care hospital in Karachi, Pakistan, from January 2013 to December 2018.

Results: We identified 58 patients with *R. terrigena* isolated from different cultures specimens, of which n=12 (22.6%) were colonizers. The median age was 61.5 years (IQR=43-71), and most were male (n=28). The most common site of infection was the respiratory tract in 28.3%, then urinary tract in 26%, and central line in 26.1%. Amongst infected cases, 37% had septic shock, 45.7% had respiratory failure.

Conclusion: *R. terrigena* is a multi-drug-resistant organism with a high mortality rate and can cause hospital-acquired respiratory tract infections in patients.

Keywords: *Raoultella terrigena, Klebsiella terrigena, Raoultella* species, *Klebsiella* species.

BACKGROUND

Raoultella terrigena, previously known as "*Klebsiella terrigena*," was discovered in 1981 and is a rare gram-negative organism, primarily found in soil and water.[1] It was distinguished from *Klebsiella* species in 2001 based on molecular analysis.[2] These organisms are oxidase negative, capsulated, immotile, facultatively anaerobic, and aerobic bacilli[2]. However, many microbiology laboratories continue to identify this organism as a "*Klebsiella species*," making it difficult to estimate its true incidence.[3] The first reported case as a human pathogen was in 2007 in a middle-aged post-liver transplant patient with endocarditis[4]. Another case report was published on sepsis secondary to *R. terrigena* in 2011[5]. A literature search reveals that most of the reported cases caused by genus *Raoultella* consist of *R. orinithinolytica* and *R.planticola*,[6,7] and are associated with diseases of the biliary tract and post-surgical interventions[6,8] as well as necrotizing fasciitis[9], soft tissue infection[10] and cystitis.[11] Our study describes the clinical presentations and outcomes of infections caused by *Raoultella terrigena*.

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MATERIAL AND METHODS

We conducted a cross-sectional study on all adults greater than or equal to 18 years of age, admitted to Aga Khan University Hospital (AKUH) between January 2013 and December 2018, who had *Raoultella terrigena* isolated from culture specimens. Since this was a case series, formal sample size calculation was not performed. We excluded patients with recurrent *Raoultella* infections and colonization. *Raoultella* infection was defined as the presence of *R. terrigena* in a clinically relevant culture specimen along with the presence of signs and symptoms fulfilling criteria for a specific infection site as defined by CDC.[12] Colonization was defined as isolation of *R. terrigena*

from culture specimen but not causing any symptoms or disease. The site of infection was defined using CDC definitions.[12] Polymicrobial infections were defined as infection with two or more bacteria considered pathogens; isolated from a clinically relevant culture specimen. Multi-drug-resistant organisms (MDR) are defined as bacteria resistant to one or more key classes of antibiotics for that organism. Source control of infection comprised of all physical measures to remove foci of infection where applicable such as removal of infected lines or drains, drainage of the liquid component of infection, surgical debridement, in order to restore optimal function of the involved area. Relapse was defined as clinical deterioration after a temporary improvement in patients with the same organism within 1 month of initial infection. Patients who fulfilled the eligibility criteria were consecutively included in the study. Demographic and other categorical variables such as age, sex, co-morbid conditions, prior history of hospitalizations, invasive procedures, presence of lines and surgical drains, use of antibiotics, hospital stay, intensive care unit (ICU) stay, and in-hospital mortality were collected from the electronic health records (EHR) on a pre-tested structured proforma. Microbiological data on cultures which included, blood, sputum, tracheal aspirates, pus, urine, pleural fluid, peritoneal fluid, cerebrospinal fluid, and tissue specimen positive for *R. terrigena* was also extracted from EHR.

The study received exemption from approval by the Aga Khan University Ethics review committee. (ERC Reference No:2019-1232-3287). Patient confidentiality was maintained and no personal identifiers were obtained. As this was a retrospective study, the committee waived the requirement of informed consent.

Specimen processing for culture was performed in the microbiology laboratory at Aga Khan University Hospital Karachi according to guidelines provided by the American Society for Microbiology. This involved sample inoculation on MacConkey, chocolate, and 5% sheep blood agar at 37 degrees Celsius for up to 48 hours. Blood for sheep blood agar was acquired by phlebotomizing sheep in the animal house at AKUH. Upon growth of lactose fermenter mucoid colonies after incubation on MacConkey agar, further biochemical tests were performed which included the utilization of citrate, production of hydrogen sulfide, detection of urease, production of indole from tryptophan, motility, and Triple Sugar Iron Test. If the organism tested negative to these tests, and gave an acidic slant over an acidic butt on the triple sugar iron test, it was further subjected to further identification. This was carried out by API 20 E, which consists of 20 biochemical tests. Antibiotic susceptibility testing was initially carried out on Mueller-Hinton agar with Kirby-Bauer disk diffusion test or Vitek-2 MS automated system. Colistin minimum inhibitory concentrations were confirmed by colistin broth microdilution, which is currently the recommended method as per Clinical and Laboratory Standards Institute (CLSI). Results were interpreted as per CLSI guidelines.

Descriptive analysis was performed for all patient-related variables with frequencies and proportions reported for categorical variables like sex, comorbid, clinical features and median with interquartile range reported for continuous variables like age, hospital stay. Chi-square test or Fisher exact test were used as appropriate to determine the association between two categorical variables, e.g., chronic kidney disease and death. IBM® Statistical Package for Social Sciences (SPSS®, version 25.0) was used for data analysis. A *p*-value of less than 0.05 was considered significant.

RESULTS

A total of 58 patients with *R. terrigena* isolated from different culture specimens were identified. Out of those, 12 isolates were identified as colonizers and excluded, remaining (46) were included in the study. The median age was 61.5 years (IQR= 43-71), with more males than females (60.9 % vs. 39.1%). The most frequent co-morbid conditions were diabetes mellitus in n=18 (39.1%) patients. Most patients n= 34 (73.9%) had a previous history of hospitalization (within the past six months) for various medical and surgical conditions, and majority, n=24 (52.17%) had at least one hospital admission. 78% patients (n=36) reported antibiotic use in last 6 months.

Out of 46 patients included in the study, 31 (67.4%) had had a prior culture of blood or other body fluid specimens growing a multidrug-resistant organism in the last six months (Table-I), while n=35 (76.1%) had a urinary catheter in place, and central lines were present in n=22 (47.8%). There were 29 patients (63%) with this infection who had a recent history (past six months) of invasive procedures.

The most common site of infection was the respiratory tract in n=13 (28.3%) patients which included sputum and tracheal aspirates, followed by urinary tract infections n=12 (26%), and bloodstream infections n=12 (26.1%) (Table-II). Respiratory failure was seen in n=21 (45.7%) patients, of which n=11 (52.4%) required mechanical ventilation and n=10 (47.6%) needed non-invasive ventilation. Of the n=17 patients, (37%) who were in septic shock due to *R. terrigena*, n=13, (76.5%) required vasopressors and n=4 (23.5%) were treated with fluid resuscitation.

The most common sources of cultures positive for *R. terrigena* were blood in 32.6%, sputum in 28.3%, and urine in 21.7% of the patients. *Monomicrobial growth of R. terrigena* was identified from n=26 (56.5%) of culture specimens, and polymicrobial growth was identified from n=20 (43.5%) culture specimens. Polymicrobial growth was most frequently seen in sputum n=7 (35%), followed by blood in n=6 (30%). The organism was highly resistant to most of the commonly used antibiotics. Carbapenem resistance was present in 91.3%, colistimethate resistance 65.2% (Table-III). In most cases, sensitivities were checked for tigecycline and fosfomycin after they were resistant to colistimethate

Out of 46 cases, eight patients were lost to follow-up, and treatment information was not available. The remaining 38 patients, n=31 patients (81.6%) received combination therapy, and n=6 (15.8%) received monotherapy. One patient died before starting treatment. Antibiotics used as empiric therapy were carbapenems n=24 (77.4%), beta-lactam/lactamase inhibitors n=14 (45.2%), vancomycin n=15 (48.4%), colistimethate n=13 (41.9%) cases. The most frequent treatment combination was carbapenem and colistemethate in n=11 (28.9%), followed by a combination of carbapenem with colistemethate and tigecycline in n=8 (21.1%) Mortality association with monotherapy was (p= 0.672), and with combination therapy (p= 0.70). Out of n=14 (36.84%) cases in whom repeat cultures for clearance needed, bacteriological clearance was achieved in n=9 (64.2%) cases. There were 23 cases that needed source control of underlying infection. It was achieved in n=12 (52.1%). Two cases (5.3%) relapsed.

The average hospital stay was a median of 11.50 days (IQR=6-22), with a median of 3 days before positive culture. Approximately n=23 (60.52%) of patients were seriously ill, requiring intensive care unit care with a median ICU stay of 6 days (IQR=4-11). In-hospital mortality recorded in n=17 (44.7%) patients.

In the subgroup analysis of factors associated with death in *R. terrigena* infections (Table-IV), it was found that chronic kidney disease (CKD) (*p* value = 0.029) and septic shock (*p* value= 0.001) were significantly associated with mortality. Also, persons with a high (greater or equal to three) Charlson- comorbidity index had increased mortality (*p* value = 0.002).

Table-I: Demographics of patients infected with *R. terrigena* (n=46).

Age in years	**Median: (61.50) IQR 43-71**
Characteristic	**n (%)**
Gender:	Male: 28 (60.9)
	Female: 18 (39.1)
Prior antibiotics (6, months)	36 (78.3)
Carbapenems	26 (72.2)
Beta lactam/ lactamase inhibitors	23 (63.9)
Glycopeptides	20 (55.6)
Colistemethate	11 (30.6)
Prior hospitalization: (6, months)	34 (73.9)
Prior MDROs (6, months)	31 (67.4)
CRE *K. pneumoniae*	13 (28.2)
MDR *Acinetobacter*	11 (23.9)
MDR *P. aeruginosa*	11 (23)
Central lines	22 (47.8)
VP shunt	1 (2.2)
Surgical drains	14 (30.4)
Urinary catheter	35 (76.1)
Recent procedures	29 (63)
Skin, soft tissues	9 (31)
Abdomen	7 (24.1)

CNS	7 (24.1)
Genitourinary	8 (27.6)
Stent placement	4 (13.8)
Chest:	4 (13.8)
Others:	6 (20.7)
Co-morbid	
Diabetes mellitus	18 (39.1)
Chronic kidney disease	16 (34.7)
Malignancy	11 (23.9)
Cerebrovascular accident	7 (15.2)
Chronic liver disease	8 (17)
Steroid therapy	2 (4.3)
Connective tissue disease	1 (2.1)

S.E., standard Error; IQR, Inter Quartile Range; M, male; F, female; CLABSI, Central Line Associated Blood Stream Infection; BSI, Blood Stream infection; VP, ventriculo-Peritoneal; MDRO, Multi Drug Resistant Organism; CRE, Carbapenem Resistant Enterobacterales; *K. pneumoniae, Klebsiella pneumoniae; P. aeruginosa, Pseudomonas aeruginosa;* MDR, Multi Drug Resistance; CNS, Central Nervous System

Table-II: Clinical features (n=46) and outcomes of *R.terrigena* (n=38).

Clinical feature	N (%)
Site of infection:	
Pneumonia	9 (19.6)
Tracheitis	4 (8.7)
Cystitis	6 (13)
Pyelonephritis	6 (13)
CLABSI	7 (15.2)
Unspecified BSI	5 (10.9)
Necrotizing fasciitis	2 (4.3)
Bed sore infection	2 (4.3)
Cellulitis	1 (2.2)
Septic arthritis	1 (2.2)
Ventriculitis	1 (2.2)
Peritonitis	1 (2.2)
Cholangitis	1 (2.2)
Clinical presentation:	
Hypotension (Systolic < 90)	17 (36.9)
Respiratory failure	21 (29.2)
Altered mental status:	25 (54.3)
Alive	21 (55.3)
Dead	17 (44.7)
Hospital stay (days)	Median (11.50) IQR=6-22
ICU stay (days)	Median (6) IQR=4-11

ICU, Intensive Care Unit.

Table-III: Drugs susceptibility of *R-terrigena*

Antibiotics	Sensitivity	Resistance	Intermediate	Unchecked
Amoxicillin clavulanate	2.2%	93.5%	2.2%	2.2%
Amikacin	19.6%	76.1%	2.2%	2.2%
Imipenem	4.3%	89.1%	2.2%	4.3%
Piperacillin tazobactam	4.3%	91.3%	2.2%	2.2%
Gentamicin	8.7%	89.1%	2.2%	
Ceftriaxone	2.2%	97.8%		
Trimethoprim sulfamethoxazole	8.7%	89.1%		2.2%
Ciprofloxacin	6.5%	91.3%		2.2%
Meropenem	8.7%	91.3%		
Colistemethate	23.9%	65.2%		10.9%
Tigecycline	30.4%	10.9%	26.1%	32.6%
Fosfomycin	15.2%	28.3%	19.6%	37%

Table-IV: Association of mortality with clinical features.

Clinical feature	Alive	Expired n	P-value n(%)
Total 38	**21**	**17**	
Non CKD	17	8	0.029
Chronic kidney disease	4	9	
Non diabetic	14	9	0.38
Diabetes mellitus	7	8	
Non CVA	19	15	0.82
Cereberovascular accident	2	2	
No lung disease	17	14	0.91
Chronic lung disease	4	3	
No CTD	20	17	0.749
Connecive tissue disease	1	0	
No CLD	15	13	0.72
Chronic liver disease	6	4	
No malignancy	12	13	0.21
Malignancy	9	4	
No steroids	19	17	0.477
Steroids	2	0	
No resp. failure	5	4	0.98
Respiratory failure	16	13	
No septic shock	19	8	<0.01
Septic shock	2	9	
Charleson's comorbidity index <3	13	2	<0.01
Charleson's comorbidity index ≥3	8	15	

DISCUSSION

In our study, most patients had hospital-acquired pneumonia due to *Raoultella terrigena*, followed by urinary tract infection. Complications identified in the majority included respiratory failure and septic shock. Our results showed a multi drug resistant susceptibility profile. The organism was found to be resistant to beta-lactams, carbapenems, and colistimethate.

Infections caused by the genus *Raoultella* have been commonly reported in older-aged immunocompromised patients; those who were suffering from malignancy or had undergone surgical interventions. Many of them developed infections of the biliary tract and had variable mortality rates.[13] In contrast, in our study most of the patients were middle aged males and the most frequent co-morbid conditions included diabetes followed by CKD and malignancy. Around two-thirds of them had recent surgical interventions. Compared to previously published case reports,[5,13,14] we had only one patient with biliary tract involvement, and we found a greater number of patients having nosocomial pneumonia and urinary tract infections.

A summary of previous reports published on *R. terrigena* is given in Table 5. It shows that while cases from most parts of the world were carbapenem sensitive, those from the Pakistani case series were carbapenem resistant. A study done on carbapenem resistant enterobacterales at the Aga Khan University showed that of 215 carbapenem resistant enterobacterales tested, 15.9% were also resistant to colistin[17] and it is of interest to note that 15% of the tested isolates were *Raoultella* species. Our study shows a similar result to both the studies mentioned above from the same locale, wherein most cases were resistant to carbapenem, and additionally, 65% were also resistant to colistin, which is often used as a last resort antibiotic in carbapenem resistant cases. This highlights the importance of using correct microbiological methods for identification and susceptibility of organisms in accordance with CLSI guidelines,[18] to ensure that correct susceptibility profiles are provided to clinicians for adequate treatment of such cases.

Like other cases reported before,[8-11, 13-17, 19-20] ours too showed that patients who acquired resistant strains of *R. terrigena* had complicated medical histories such as diabetes, previous hospitalization, and/or antibiotic use, invasive procedures or cultures positive for (other) resistant organisms in the last six months. In addition, our study also showed that several patients had indwelling devices in place, pointing towards the propensity of these infections being hospital acquired. Further studies, of course, are required to confirm this

hypothesis. In contrast to the many case reports we found that showed intra-abdominal infections with *R. terrigena*, our study showed pneumonia as the predominant clinical presentation.

Interestingly, we found no difference of outcome in treating patients with monotherapy or combination therapy. Literature suggests treating carbapenem and colistin resistant enterobacterales with a combination of more than two antibiotics[21] but our study showed no difference when the mortality of patients treated with monotherapy and combination therapy was compared.

STRENGTH AND LIMITATIONS

Although our study has a limited sample size and is a single-center experience, it is the largest cohort of patients reported of this rare gram-negative infection to date. Because of the scarcity of available resources, we could not identify *R. terrigena* by molecular analysis (standard method).

CONCLUSION

Infections caused by *R. terrigena* are highly drug-resistant, mostly causing hospital-acquired respiratory tract infections that are difficult to treat, leading to prolonged hospital and ICU stays with a high mortality rate. Patients with underlying renal dysfunction, those on vasopressor support, and a high CCI score are at greater risk of death due to this infection. Patients with multiple comorbid conditions and immunosuppression are at risk of acquiring infections with opportunistic organisms, including *R. terrigena*, leading to increased mortality if not identified and treated correctly.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Ishfaque Ahmed: Drafting of article, acquisition of data: laboratory or clinical, analysis of data, critical revision, final approval of manuscript, accountable for all aspects of work

Nosheen Nasir: Conception and design of study, Drafting of article, final approval of manuscript, accountable for all aspects of work

Fizza Farooqui: Analysis of data, critical revision, drafting of article, final approval of manuscript, accountable for all aspects of work

Syed Faisal Mahmood: Conception and design of study, Drafting of article, final approval of manuscript, accountable for all aspects of work

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