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Isolation and Identification of Multidrug-Resistant *Raoultella terrigena* as a Causative Agent of Urinary Tract Infection in Pregnant Women in the South of Libya

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Abstract

Raoutella terrigena is an opportunistic bacteria that rarely cause infections in humans. It is facultative Gram negative, rod shaped, present mostly in water, plants, soil, fish and insects. Two strains of *R. terrigena* have been isolated during a study searching of the must causative bacteria of urinary tract infection, in pregnant women where it counted about 2% of the total isolates. Susceptibility test has revealed multi drug resistance to about 61.5 % of the used antibiotics. This is the first documented report of such infection in Libya.

Keywords: *Raoutella terrigena*, Urinary tract infection, Libya, Pregnant women. Antibiotic resistance

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Introduction

Raoultella terrigena, which is formally known as *Klebsiella terrigena* and closely linked to genus *Klebsiella*, is belonging to *Enterobacteriaceae* family (Izard, *et al.,* 1981; Drancourt, *et al.,* 2001). These organisms are Gram negative, oxidase negative, non-motile, non-spore-forming, facultative anaerobic, and capsulated bacilli (Podschun and Ullmann, 2000; Drancourt, *et al.,* 2001; O' Connell and NiRiain, 2010)

The discovery of this bacterium as *Klebsiella terrigenna* was introduced by Izard, et al. in 1981, however in 2001 based on molecular analysis 16S *rRNA* sequences and *rpoB* genes Drancourt and his colleagues have separated *Raoultella* from *Klebsiella* species (Drancourt, *et al.,* 2001). This finding lead to the establishment of the new genus *Raoultella* which consists of four species: *Raoultella terrigena*, *Raoultella ornithinolytica*, *Raoultella planticola*, and *Raoultella electrica* (Chun, *et al.,* 2014; Tantasuttikul and Mahakarnchanakul, 2019) .

These species are mainly found in water, plants, soil, fish, and insects (Podschun and Ullmann, 2000; Yu, *et al.,* 2011; Sandal and Ozen, 2014; Mal, *et al.,* 2019). Occasionally, with a low prevalent rate of infection, the members of this genus have been recognized as a human opportunistic pathogen and have the capability of causing diseases (Drancourt, *et al.,* 2001; Chun, *et al.,* 2014). *R. terrigena* has been reported to harbour several virulence factors that have been found in Klebsiella pneumonia, however very few cases of infections by this species have been reported (Podschun and Ullmann, 2000; Ahmed, *et al.,* 2020; Lekhniuk, *et al.,* 2021).

The first documented case of *R. terrigena* as a human pathogen was confirmed in 2007 in a patient with a post-liver transplant and endocarditis (Goegele et al., 2007). This bacterium has been isolated from other different clinical sites such as subungual abscess**,** bronchial secretion, blood and urine **(**Wang, *et al.,* 2016; Mal, *et al.,* 2019; Ahmed, *et al,.* 2020; Lekhniuk, *et al.,* 2021).

Until now, a total of 363 cases of *R. terrigena* have been documented, and of these about 38.6% were found to show a multidrug resistant profile (Lekhniuk, *et al.,* 2021). In this study we document the first report of the isolation of two multi drug resistance (MDR) *R. terrigena* strains from urine specimens in Libya, South region, Wadi Alshati.

Material and methods:

Microbial isolation and culture:

Urine sample was examined by urinalysis and urine culture. For urinalysis, dipstick tests using Comber 10 reagent test strips were used, and wet smear preparations were made from sediment of each urine sample after centrifugation and were microscopically examined at x40 for detection of

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white blood cells as an indicator of pyuria. Samples with ≥ 10 WBC/field were regarded as pyuria.

For urine culture, a quantity of urine was taken using a loop (0.01 ml) and cultured on Petri dishes containing Blood agar (Oxiod, UK) and McConkey agar (Oxiod, UK), and the dishes were incubated at 37°C for 24-48 hours. After the incubation period, bacterial growth on the three media was examined, bacterial colonies were counted, colonies were described. Taking into account the number of colonies, urinary tract infection (UTI) was considered as positive when growth of ≥100,000 Colonies Forming Unit (CFU) per ml in urine sample culture is detected.

Biochemical identification:

In this assay the fresh pure culture of isolated pathogens has been used. The pathogen isolates were identified using the API 20E kit (bio-Mérieux), according to the manufacturer's instructions and *E. coli* ATCC 25922 was used as a quality control strain for API 20E test. The resultant phenotypic profiles were compared to the bio-Mérieux online database, version 3.0, at https://apiweb.biomerieux.com.

Antibiotic sensitivity test:

The assay was performed, on Muller-Hinton agar, by using disk diffusion method following the instructions in guidelines, version 12 – May 2013, described by British Society for Antimicrobial Chemotherapy (BSAC, 2013). The antimicrobial susceptibility test disks (MAST Group Ltd, UK) included Ampicillin 10µg, Amoxicillin 25µg, Amoxicillin/clavulanic acid 30µg (Augmentin), Ceftazidime 30µg, Ceftriaxone 30µg, Cefuroxime 30µg, Amikacin 30µg, Ciprofloxacin 5µg, Gentamycin 10µg, Imipenem 10µg, Nitrofurantoin 200µg, Ticarcillin 75µg, Pipercillin 30µg. Type strain *E. coli* ATCC25922 was used in this test as a reference strain.

Result and discussion:

The occurrence of *Raoultella terrigena* infections is known to be a very rare, however some studies have reported the isolation of *R. terrigena* from several clinical sites, such as urinary tract (Wang, *et al.*, 2016; Mal, *et al.*, 2019; Ahmed, *et al.*, 2020; Lekhniuk, *et al.*, 2021). Until the date of this study, just about 363 cases of *R. terrigena* infections have been published in the world, while according to our knowledge there is no reported cases either in Libya or Arabic countries (Lekhniuk, *et al.*, 2021).

Two *R. terrigena* strains which are accounting about 2% of the total isolates have been identified during this study among other isolates. Other genus were also recognized in this study include; *Staphylococcus* coagulase-positive (33.65%), *Staphylococcus* coagulase negative (25.96%),

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Streptococcus sp (23.08%), *Enterobacter cloacae* (6%), E. coli (3%), *Klebsiella pneumonia* (2%), *Enterobacter cancerogenus* (1%), *Salmonella* sp (1%), *Pseudomonas lutola* (1%), *Proteus mirabilis* (1%), and *Citrobacter sediaki* (1%). Nevertheless, this study focuses mainly on *R. terrigena*.

The specimens were firstly investigated by general urine analysis and then cultured on Blood and MacConkey agar. Both specimens showed to be full with white blood cells which indicates the present of UTI. After 24 hours of incubation, the colonies appeared as lactose fermenter mucoid colonies on MacConkey agar (figure 1), while smooth, circular, light yellow, and non-hemolysis on Blood agar. The isolated organisms were subjected to Gram straining and microscopic technique revealed the presence of Gram negative short rod bacteria (figure 2).

Further biochemical identification, Api20E (bioMérieux) was carried out in order to identify the type of isolated Gram negative rod. The api20E scheme detect the two strains as *R. terrigena* with 90% and 93% for both isolated bacteria, though, molecular techniques such as 16s RNA assay is a golden standard method for identification of pathogenic bacteria. This study could not conduct any genetic identification technique due to scarcity of available resources. However, the identification characteristics showed in this study were previously reported in a limited number of published papers confirming that our finding can be reliable (Mal, et al. 2019; Ahmed, et al. 2020).

Interestingly, all used biochemical tests except three showed same reaction for both *R. terregina* and *K. pneumonia* isolates. The exception was Urea (URE), inositol (INO) and amygdalin (AMY) tests, which showed negative reactions for *R. terregina* and positive reactions for *K. pneumonia* strains (Table 1). The responsible enzymes for utilizing such molecules are urease, INOsitol and AMYgdalin respectively (Biomanufacturing, 2023). Such enzymes are required by the bacteria to break down the targeted substances, however the used *R. terregina* strains seem to have no ability for this mechanism. Thus, the negative reactions for the above mentioned three tests allowed the api 20E scheme to distinguish *R. terregina* from *K. pneumonia*.

The two *R. terrigena* strains in this study have been isolated from pregnant women who were 22 and 30 year old. Both were complaining from symptoms of urinary tract infections and were under the prescription of antibiotics, Augmentin and Ampicillin, without antibacterial susceptibility test (Table 2). The outcomes of given antibiotics were not same, Augmentin was effective and the first patient was cured in a week of antibiotic course, while the use of Ampicillin did not assist in relieving the UTI symptoms for second patient (Table 2).

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Accordingly, antibiogram was performed in order to recognize the susceptibility of the two isolated *R. terrigena* strains to some antimicrobials agents that used in the study area as shown in table 3. Certain studies investigated the susceptibility ability of *R. terrigena* and one of these studies conducted by Wang et al. in 2016 revealed that *R. terrigena* was sensitive to the nearly all used antibiotics, except ampicillin (Wang et al., 2016). However, other more recent studies confirmed the presence of previous history of multidrug-resistant of this bacterium (Mal *et al.*, 2019; Ahmed, *et al.*, 2020; Lekhniuk, *et al.*, 2021).

Indeed, in the current study, the two isolates of *R. terrigena* were found to be resistant to about 61.5% of the used antibiotics, nevertheless these two strains are still sensitive to the rest of the used antibiotics which are Ceftazidime, Cefuroxim, Augmintin, Ciprofloxacin, and Nitrofurantoin. Interestingly, the finding of sensitivity test of Augmentin and Ampicillin was in constant with the treatment outcomes after taken the prescribed antibiotics by the two patients, as mentioned above. Similarly, in the sensitivity test both strains were sensitive to Augmentin but were resistant to Ampicillin (Table 3).

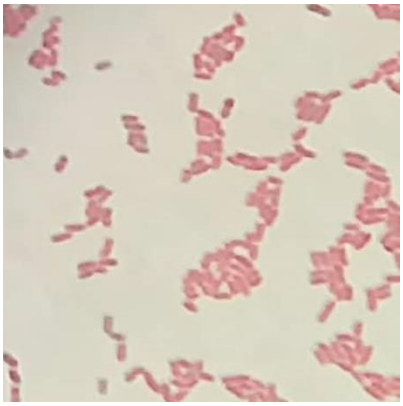

Figure 1. Gram stain of *R. terrigena* cells

Figure 2. Colonies growths of *R. terrigena* on MacConkey agar.

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Table 1. API 20E profile results after 48 hours of incubation

Biochemical Tests	Isolate1	Isolate2	Isolate3	Isolate4
ONPG	+	+	+	+
ADH	+	+	-	+
LDC	+	+	+	+
ODC	-	-	-	-
CIT	+	+	+	+
H2S	-	-	-	-
URE	-	-	+	+
TDA	+	+	+	+
IND	-	-	-	-
VP	+	+	+	+
GEL	-	-	-	-
GLU	+	+	+	+
MAN	+	+	+	+
INO	-	-	+	+
SOR	+	+	+	+
RHA	+	-	+	+
SAC	+	+	+	+
MEL	+	+	+	+
AMY	-	-	+	+
ARA	+	+	+	+
OX	-	-	-	-

1= R. terregina 1, 2= R. terregina2, 3= K. pneumonia1, 4 K. pneumonia2.

Table 2. Clinical details of the two patients

Cases	Age	Symptoms	Pyuria	Antibiotic	AST	Outcome
Patient 1	22	Yes	+	Augmentin	No	Cured
Patient 2	30	Yes	+	Amoxicillin	No	Not cured

Table 3. Susceptibility pattern of *Rouatella terrigena* to the antimicrobial agents

Type of antibiotic	**Resistance**	**Sensitive**
Ampicillin	2(100%)	0%
Imipenem	2(100%)	0%
Ticarcillin	2(100%)	0%
Pipercillin	2(100%)	0%
Ceftriaxone	1(50%)	1(50%)
Ciprofloxacin	0%	2(100%)
Gentamicin	2(100%)	(0%)
Amikacin	2(100%)	0%
Amoxicillin	2(100%)	0%
Nitrofurantoin	0%	2(100%)
Augmentin	0%	2(100%)
Ceftazidime	0%	2(100%)
Cefuroxime	0%	2(100%)

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Conclusion

The rate of *R. terrigena* infections is expected to be underestimated due to the lack of documented reports of these infections. Therefore, the real prevalence rate of *R. terrigena* infections might be more than we expect. The importance of *R. terrigena* infections have been increased since antimicrobial resistance was detected among this bacteria. Our current report is an additional evidence for the clinical significant of *R. terrigena*. This report rises a call for more studies to be focused on this potential opportunistic pathogen, in the future. This study recommend also the use of molecular genetic methods to be introduced in routine and research work in the hospitals and clinics in Libya to detect the infections of *R. terrigena*.

Conflict of Interest: The authors reported no conflict of interest.

Data Availability: All of the data are included in the content of the paper.

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