



KOCURIA KRISTINAE IS AN EMERGING CAUSATIVE AGENT OF RECURRENT URINARY TRACT INFECTIONS IN IRAQI WOMEN

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Abstract

Based on some cultural and biochemical tests in addition to VITEK compact 2 system, 64 bacterial isolates distributed on 25 species were isolated from these cases, interestingly, *Kocuria kristinae* constituted the highest isolation percentage (13 isolates; 20.3%), followed by *Staphylococcus haemolyticus* and *Escherichia coli* (8 isolates; for each). To our best knowledge, it is the first time that this bacterial species implicated in UTI in Iraq.

Key words : recurrent UTI, *Kocuria kristinae*, Iraqi women.

Introduction

A symptomatic urinary tract infection (UTI) that subsequent a clinically recovered earlier episode of UTI, generally yet not inevitably, after appropriate treatment, referred to as a recurrent UTI. Nonetheless, it alludes to more than one infection in a half year more than two infections per year (Hooton, 2001). These infections are furthest frequently seen in young and adult females and are related with colonization by the faecal flora via ascending route from. Infection occurs through bacterial colonization of the vagina and distal urethra, which subsequently ascends into the bladder (Epp *et al.*, 2010). Reservoirs of bacteria may remain in the vagina and gastrointestinal tract of susceptible patients (Aydin *et al.*, 2014). Frequently, the etiologic agents of recurrent UTI are similar to that of sporadic infection. The majority of uropathogens are rectal flora ascending to the bladder; subsequently, colonising the periurethral area as well as urethra. Additional important UTI causing-uropathogens may embrace *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis* and *Klebsiella pneumoniae*. Nonetheless, *Klebsiella* and group B streptococci infections are highly encountered in diabetic patients. Furthermore, infections due to *Pseudomonas* spp. are most frequent in patients with prolonged catheterisation (Gupta *et al.*, 2011).

Due to the shortage of investigations related to recurrent UTI in Iraqi women, this work was designed to prevail the implicated bacterial species.

Materials and Methods

Ethical statement

All participants agreed to provide the investigator with mid-stream urine specimens. Informed consent according to the Declaration of Helsinki was obtained from all participants

Study group

A total of 104 non-pregnant Iraqi women were enrolled in the current study. These women were presented with recurrent UTI referring several hospitals in Baghdad: Al-Yarmouk, Al-Imam Ali, and Saint Raphael (Al-Rahibat) during the period 1/12/2018 to 1/5/2019. An individual was considered as a recurrent UTI patient when she suffered three UTIs with three positive urine cultures during 12 months, or two infections during the previous 6 months. Each case was validated by the hospital urologist.

Specimens collection

Mid-stream urine specimens were collected in a sterile wide-mouth container. The participant was instructed to clean the genitalia carefully before urine voiding.

Isolation and Identification of bacteria

All specimens were culture onto blood agar, mannitol

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salt agar and MacConkey agar and incubated at 37°C for 24 hr. The grown colonies were submitted to Gram stain technique; thereafter, identified with conventional biomedical and cultural methods (Harley, 2016). The identification was confirmed using VITEK 2 compact system (bioMérieux, France).

Results and Discussion

About one hundred and two different clinical specimens were collected from non-pregnant women aged between 18 – 49 years who attending hospitals in Baghdad. Mid-stream urine specimens were cultured on Blood agar, Nutrient, MacConkey and Mannitol salt agar. The results revealed that 64 (62.74%) women has a positive bacterial infection.

On blood agar, the region appears lightened (yellow) and translucent due to complete lysis of red cells in the media around and under the colonies, 17 (27.41%) isolates formed colonies surrounded by beta-hemolysis zone. Hemolysin is an exotoxin formed by *S. aureus* that causes whole red blood cell lysis (Sritharan, 2006).

Moreover, 23 (37%) isolates gave positive result on MacConkey agar by showing pink colonies which is an

indicator for lactose fermentation, and 39 (62%) isolates developed a growth on mannitol salt agar; one (1.61%) of them was mannitol fermenter seen as yellow colonies.

Thereafter, the resultant colonies were submitted to Gram stain. The results revealed that 41 (64.1%) and 23 (35.9%) isolates showed Gram-positive and Gram-negative stainability, respectively.

According to previous findings, isolates submitted to VITEK 2 compact automated system and the results are demonstrated in Table 1. The result revealed that 64 individual had bacterial infection exemplified by 25 species. Of interest, *Kocuria kristinae* was the dominant species (13 isolates; 20.3%).

Markedly, many rare UTI causing bacterial species were isolated in this study. More likely, the reason laid behind may mainly be owing to the accuracy of the identification method employed in this work. Moreover, the difference in the study population and the nature of the infection itself could have a part in such isolation results.

Kocuria kristinae can cause numerous opportunistic infections in patients associated with medical indwelling devices and intense underlying illnesses (Tewari *et al.*, 2013). Citro *et al.*, (2013) demonstrated that *K.*

kristinae is the causative agent associated with catheter-related bacteraemia, and they likely owed the reason that *Kocuria* species are typical skin resident.

While there are currently numerous traditional microbial identification systems using phenotypic methods that can provide an inaccurate result for coagulase-negative organism particularly *Staphylococcus epidermidis*, leading to misidentification of *Kocuria* organism. While the accurate identification of *Kocuria* spp. require more sophisticated techniques to be verified (Ben-Ami *et al.*, 2005).

Boudewijns *et al.*, (2005) emphasized the reliability of Vitek 2 system as an identification technique for *K. kristinae*. Likewise, the use of this technology in the routine practice may result in improved comprehension of the potential pathogenicity of *K. kristinae*.

Layer *et al.*, (2006) demonstrated the good performance of the VITEK 2 technology, permitting their use in routine microbiology laboratory with a high level of identification accuracy. Yet, when clear-cut identification of distinguished *Staphylococcus* spp. is essential, molecular identification remains the most accurate and reliable method of identification.

Table 1: Bacterial species isolated from females with recurrent urinary tract infection.

Id	Bacterial species	Number	Percentage
1	<i>Kocuria kristinae</i>	13	20.3
2	<i>Staphylococcus haemolyticus</i>	8	12.5
3	<i>Escherichia coli</i>	8	12.5
4	<i>Staphylococcus arletta</i>	4	6.3
5	<i>Klebsiella pneumoniae</i>	4	6.3
6	<i>Dermacoccus nishinomiyaensis</i>	4	6.3
7	<i>Staphylococcus hominis</i>	2	3.1
8	<i>Staphylococcus epidermidis</i>	2	3.1
9	<i>Kocuria rosea</i>	2	3.1
10	<i>Granulicatella elegans</i>	2	3.1
11	<i>Staphylococcus equorum</i>	1	1.6
12	<i>Staphylococcus aureus</i>	1	1.6
13	<i>Sphingomonas paucimobilis</i>	1	1.6
14	<i>Serratia ficaria</i>	1	1.6
15	<i>Pseudomonas stutzeri</i>	1	1.6
16	<i>Pseudomonas putida</i>	1	1.6
17	<i>Pseudomonas fluorescens</i>	1	1.6
18	<i>Pantoea agglomerans</i>	1	1.6
19	<i>Micrococcus lylae</i>	1	1.6
20	<i>Micrococcus luteus</i>	1	1.6
21	<i>Klebsiella oxytoca</i>	1	1.6
22	<i>Comamonas testosteroni</i>	1	1.6

This bacterium is usually identified wrongly as coagulase-negative staphylococci (CONS) in clinical microbiology laboratories based on its Gram stainability, catalase and coagulase activity. Moreover, *Kocuria* has another physiological and biochemical properties including the development of non-hemolytic colonies on the agar non-capsulated, non-spore forming, non-motile, non-acid fast and positive for Voges-Proskauer test (VP). Likewise, it has been noticed that the different species of *Kocuria* respond differently to routine biochemical tests such the oxidase, amylase, urease, citrate utilization test, gelatinase, phosphatase tests, utilization of inulin, arabinose, N-acetyl-L-glutamic acid, and nitrate reduction tests (Savini *et al.*, 2010). This might be due to the explanation behind the inaccurate identification by both traditional and automated bacterial recognition systems (Kandi *et al.*, 2016).

Conclusion

Many bacterial species have been isolated from Iraqi women with recurrent UTI; however, *K. kristinae* was the predominant species

References

- Aydin, A., K. Ahmed, I. Zaman, M.S. Khan and P. Dasgupta (2014). Recurrent urinary tract infections in women. *Int. Urogynecol. J.*, **26**: 795-804.
- Ben-Ami, R., S. Navon-Venezia, D. Schwartz, Y. Schlezinger, Y. Mekuzas and Y. Carmeli (2005). Erroneous reporting of coagulase-negative *Staphylococci* as *Kocuria* spp. by the Vitek 2 system. *J. Clin. Microbiol.*, **43**: 1448-50.
- Boudewijns, M., J. Vandeven, J. Verhaegen, R. Ben-Ami and Y. Carmeli (2005). Vitek 2 automated identification system and *Kocuria kristinae*. *J. Clin. Microbiol.*, **43**: 5832; author reply 5832.
- Citro, R., C. Prota, L. Greco, M. Mirra, A. Masullo, A. Silverio, E. Bossone and F. Piscione (2013). *Kocuria kristinae* endocarditis related to diabetic foot infection. *J. Med. Microbiol.*, **62**: 932-934.
- Epp, A., A. Larochelle, D. Lovatsis, J.E. Walter, W. Easton, A. Epp, S.A. Farrell, L. Girouard, C. Gupta, M.A. Harvey, A. Larochelle, M. Robert, S. Ross, J. Schachter, J.A. Schulz, D. Wilkie, W. Ehman, S. Domb, A. Gagnon, O. Hughes, J. Konkin, J. Lynch and C. Marshall (2010). Recurrent Urinary Tract Infection. *Journal of Obstetrics and Gynaecology Canada*, **32**: 1082-1090.
- Gupta, K., T.M. Hooton, K.G. Naber, B. Wullt, R. Colgan, L.G. Miller, G.J. Moran, L.E. Nicolle, R. Raz, A.J. Schaeffer, D.E. Soper, A. Infectious Diseases Society of M. Society for and D. Infectious (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis.*, **52**: e103-20.
- Harley, J.P. (2016). *Laboratory Exercises in Microbiology* 10th ed. New York: McGraw-Hill Higher Education.
- Hooton, T. M. (2001). Recurrent urinary tract infection in women. *Int. J. Antimicrob. Agents*, **17**: 259-68.
- Kandi, V., P. Palange, R. Vaish, A.B. Bhatti, V. Kale, M.R. Kandi and M.R. Bhoomagiri (2016). Emerging Bacterial Infection: Identification and Clinical Significance of *Kocuria* Species. *Cureus*, **8**: e731.
- Layer, F., B. Ghebremedhin, K.A. Moder, W. Konig and B. Konig (2006). Comparative study using various methods for identification of *Staphylococcus* species in clinical specimens. *J. Clin. Microbiol.*, **44**: 2824-30.
- Savini, V., C. Catavitello, G. Masciarelli, D. Astolfi, A. Balbinot, A. Bianco, F. Febbo, C. 'Amario and D. D'Antonio (2010). Drug sensitivity and clinical impact of members of the genus *Kocuria*. *J. Med. Microbiol.*, **59**: 1395-1402.
- Sritharan, M. (2006). Iron and bacterial virulence. *Indian J. Med. Microbiol.*, **24**: 163-4.
- Tewari, R., M. Dudeja, A.K. Das and S. Nandy (2013). *Kocuria kristinae* in catheter associated urinary tract infection: a case report. *Journal of clinical and diagnostic research: JCDR*, **7**: 1692.