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Isolation, Identification and Evaluation of Antibacterial Resistance in Kocuria Kristinae Isolated from Pneumoniae Patients in Hospitals in Iraq

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ABSTRACT
Kocuria kristinae is a bacterium classified as a gram-positive aerobic, and it is a member of the normal flora of skin and is uncommon source of infection. This study aimed to isolate and identify K. kristinae from pneumonia patients in Iraqi hospitals and evaluate their antibiotic resistance profiles. The current study was conducted to determine K.kristinae clinical isolates taken from many hospitals in in Babil province،Iraq. A total of 150 different clinical samples were obtained. A total 93 isolates were obtained from clinical specimens have growth, 42 isolates (45.16%) were confirmed as K. kristinae using biochemical tests and the VITEK 2 system. 39 Gram negative bacteria, 7 fungi and 5 mix between G+ve and G-ve while other hand, 57 samples have no growth on culture media. The antibiotic susceptibility results showed by VITEK automated system in detecting antimicrobial susceptibility patterns among isolates, its revealed that 23 K.kristinae isolates (54.8%) were found be sensitive to Gentamycin, linezolid, Tetracycline, tigecycline, Rifampicin, Doxycycline and Ciprofloxacin, in other hands, 18 isolates (42.8%) were resistant to Benzylpencillin, Moxifloxacin , meropenem and Fucidic acid and 1 isolate (2.4%) was intermediate to one antibiotics (Vancomycin). Documented cases of infections caused by Kocuria spp. are limited. Most of these infections have been detected in hospitalized patients with severe underlying diseases or who had indwelling devices or implants or suppressed immunity. These findings highlight the potential clinical significance of K. kristinae and the importance of monitoring its antibiotic resistance patterns.

KEYWORDS
Kokuria kristinae infection, pneumonia, antibiotic resistance, VITEK 2, opportunistic pathogens

INTRODUCTION

Kocuria are Gram-positive, coccoid actinobacteria that occur in tetrads belonging to the family Micrococcaceae, suborder Micrococcineae, order Actinomycetales (Stackebrandt et al.,1995). They are widely distributed in nature and can also be found frequently as normal skin and oral cavity flora in humans and other mammals. The genus contains 18 species ,only five of which are known to be opportunistic pathogens (Savini et al.,2010). Kocuria spp. is ubiquitous in various natural habitats and frequently isolated as normal skin flora in humans and other mammals. *K. kristinae* can be described as a facultative anaerobic, non-motile, catalase positive, coagulase negative gram-positive coccus which is baled typically in tetrads. Infections from *Kocuria spp*. have been poorly documented. Of the few reported cases, it

was identified infection in hospitalized patients with some significant preexisting conditions, those possessing indwelling devices or implants, and also immunosuppressed patients. Like other *Kocuria* spp, *K. Kristinae* was not considered to be a primary pathogen, but during recent years, well documented cases of catheter related bacteraemia which was caused by this species in chronically ill patients (Lai et al.,2011).

Although *K. kristinae* is not commonly recognized as a primary pathogen, recent studies have identified it as an opportunistic microorganism associated with various infections, especially in immunocompromised or hospitalized patients. The genus Kocuria, which comprises 18 species, includes only a few that are pathogenic, with *K. kristinae* being one of the more clinically significant species in recent years.

Historically, *K. kristinae* has been considered a benign commensal, with its role in human infections remaining largely unexplored until relatively recently. Infections caused by K. kristinae are often reported in patients with severe underlying diseases, those with indwelling medical devices, or individuals who are immunocompromised. Documented cases of infection are relatively rare but have been increasingly noted in clinical settings, particularly in cases of catheter-related bloodstream infections, infective endocarditis, urinary tract infections, and pneumonia. Despite this*, K. kristinae* remains underrepresented in clinical microbiology studies, and its potential for causing infections in susceptible individuals is not fully understood.

Furthermore, the increasing occurrence of multidrug resistance in *K. kristinae* isolates has raised concerns about the effectiveness of conventional antibiotic therapies, highlighting the need for further investigation into its pathogenicity and antimicrobial resistance profiles. This study, therefore, aims to provide a comprehensive analysis of *K. kristinae* as an emerging pathogen and to contribute to the understanding of its clinical significance, particularly in the context of pneumonia infections in hospitalized patients.

METHODS

Sampling and study design: This cross-sectional study was carried out to feature K. Kristinae isolates gotten from big hospitals in Babylon province, Iraq. A total of 42 different clinical specimens (urine, sputum, and blood) taken from patients who came to the out-patient clinics in Babylon province, Iraq was collected within the time frame of January 2024-March 2024. All patients gave verbal consent before sampling.

Isolation and Identification of *K. kristinae*: Clinical samples was cultured on MacConkey agar and Blood agar. The identification of isolates to the species level was through biochemical tests oxidase, catalase, and the Vitek 2 system. The classification of microbes isolated and identified was based on shape as well as colony color for each type of agar on which they grew. Also done was Gram staining plus a look under a microscope.

Determination of antibiotic susceptibility patterns:

Antibiotic susceptibility testing was done using the automated VITEK system bioMérieux, following the manufacturer's instructions. The clinical isolates were inoculated into the appropriate VITEK cards, and susceptibility to a panel of antibiotics was determined. The antibiotics tested included Gentamicin, Linezolid, Tetracycline, Tigecycline, Rifampicin, Doxycycline, Ciprofloxacin, Benzylpenicillin, Moxifloxacin, Meropenem, Fusidic acid, and Vancomycin. The results were interpreted automatically by the VITEK software based on established Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS

Isolation of K. kristinae: Results of distribution of different clinical samples showed that most samples obtained , were from urine 23 (54.76%) while other samples distributed among samples of sputum and blood(table 1). K. kristinae was isolated and identified based on biochemical tests and Vitek 2 system, as well as cultural properties on blood (fig 1) and MacConkey agar medium plus gram stain in lab . Out of 150 clinical samples , out of 93(62 %) that showed positive cultures ,only42 isolates ;45.16 % belonging to K. kristinae.

Table 1: Distribution of *K. kristinae* isolates among different clinical samples

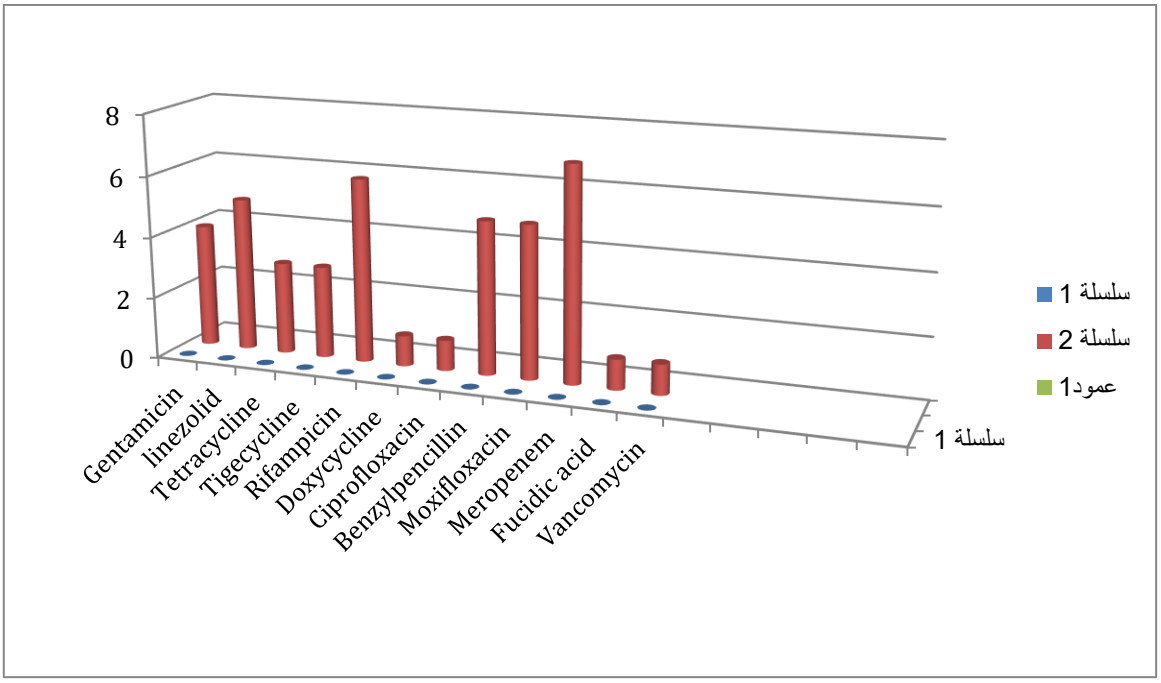
Clinical sample type	No. (%) samples	No. (%) of *K. kristinae* isolates
Urine	23	54.76%
Sputum	16	38.10%
Blood	3	7.14%
Total	42	100%

Fig (1): *K. kristinae* on blood agar

Table (1-2): Antibiotic Susceptibility of *K. kristinae* isolates:

Antibiotics	MIC	No.
Gentamycin	**S**	**4**
linezolid	**S**	**5**
Tetracycline	**S**	**3**
Tigecycline	**S**	**3**
Rifampicin	**S**	**6**
Doxycycline	**S**	**1**
Ciprofloxacin	**S**	**1**
Benzylpencillin	**R**	**5**
Moxifloxacin	**R**	**5**
meropenem	**R**	**7**
Fucidic acid	**R**	**1**
Vancomycin	**I**	**1**
Total		**42**

Abbreviations: S, susceptible; R, resistant; I, Intermediate: MIC, minimal inhibitory concentration (mcg/ml). Antibiogram interpretation according to EUCAST 2021 guidelines.



DISCUSSION

K. kristinae was first described in 1974 and is noted to be catalase-positive, coagulase-negative, non-motile, and gram-positive facultative anaerobe with typical tetrad formation. It can act as a natural commensal microorganism on the skin and mucosal flora of several mammals but is an opportunistic pathogen. Of the 18 described species in the genus Kocuria, five are reported pathogenic (Hassan et al., 2016). In our study, isolation of Kocuria kristinae from various clinical samples constituted 23 (54.76)% of total isolates. This organism was found to grow on blood agar and nutrient agar but not on MacConkey's agar medium. Gram staining showed growth as gram-positive cocci mostly in tetrads. It was catalase positive. It also gave a positive response for some sugars like glucose (AGLU), maltose (dMAL), sucrose (SAC), lactose (LAC) and optochin (OPTO) in the Vitek biochemical profile, with 89% probability identification. These characteristics are not typical of this strain among micrococci, and thus its biochemical reactions include utilization of these sugars and fermentation of lactose, as well as being optochin resistant. In an investigation carried out in Switzerland by Gayral et al. during 1997, the Vitek 2 system was tested for its performance towards the rapid identification of 845 strains of medically significant gram-negative rods. Only in 7 strains did misidentification occur (0.8%), while in 10 strains identification did not occur at all(1.2%). The relatively high isolation rate of K. kristinae in this study, as compared to the several studies mentioned earlier, could be due to the fact that this investigation focused on preselected samples that are generally acknowledged as common infection sites for this particular bacterium. In recent years, *K .kristinae* was reported as a primary pathogen in cases of catheter related bacteraemia and infective endocarditis (Lai *et al*.,2011) bacteremia with acute leukemia (Martinaud *et al.,*2008) pregnant female (Dunn *et al.,* 2011) and acute cholecystitis (Ma *et al.,*2005). In the recent reported cases by Tewari et al., (2013) of *K. kristinae* in urinary tract infection in a catheterized, 20-years old male that was considered as the first reported case of a catheter related urinary tract infection which was caused by *K. kristinae*. In another report in India by Lakshmikantha et al., (2015) they isolated *K. kristinae* from urine and blood samples. The resistance observed to commonly used antibiotics such as Meropenem and Moxifloxacin underscores the growing concern of multidrug resistance in opportunistic pathogens. Notably, over 50% of isolates were susceptible to multiple antibiotics, suggesting some therapeutic options remain viable. The findings support the need for routine identification and susceptibility testing of *Kocuria* spp. in clinical settings. There are reports on the false identifications of coagulase-negative Staphylococci as Kocuria spp. by the VITEK 2 system due to its phenotypic variability (CLSI, 2008) Due to lack of facilities, we did not confirm our isolate of *K. kristinae* by genotyping. We believe that modern VITEK 2 compact automated system with a GP card which was covered by the corresponding database, was quite a reliable tool for identification of *Kocuria kristinae* in our patient (Boudewijns, 2005).

Conclusion: This study demonstrates the emergence of *Kocuria kristinae* as an opportunistic pathogen among pneumonia patients in Iraq. The bacterium showed diverse resistance patterns, emphasizing the need for proper diagnostic identification and susceptibility testing. Gentamycin, Linezolid, and Rifampicin may be effective treatment options. Ongoing surveillance and molecular studies are recommended to better understand the resistance mechanisms and clinical behavior of this organism.

Ethical Clearance: The study was conducted following the ethical principles which have their origin in the Declaration of Helsinki. A local Ethics Committee reviewed and approved the study protocol as well as the subject information and consent form

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