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COMBATING KOCURIA: A GAME OF SHADOWS

Microbiology

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ABSTRACT

INTRODUCTION: Kocuria species is now an upcoming pathogen. They are gram-positive cocci and are catalase positive but coagulase negative, hence causing confusion with coagulase negative staphylococci. There are no CLSI guidelines for antibiotic sensitivity testing yet established for this pathogen. Here we present some cases in the pediatric age group caused by Kocuria spp.

MATERIALS AND METHODS: Kocuria spp. were isolated from 28 different clinical samples. The morphological and biochemical features suggestive of Kocuria were confirmed by Vitek-2 system. Susceptibility testing was performed according to the CLSI guidelines for Staphylococcus.

RESULTS: We found four Kocuria spp. causing infections – K. kristinae, K. rosea, K. varians and K. rhizophila. Out of 28 isolates, 13 were multidrug resistant.

CONCLUSION: Insufficient literature is available to establish antibiotic susceptibility profile of this bacteria. Available data show it to be broadly susceptible to most of the antibiotics. However, the present study showed a different picture.

KEYWORDS

Kocuria, Pediatrics, Identification, Antibiotic Susceptibility

INTRODUCTION

Kocuria species has now emerged as a pathogen, especially in the compromised hosts. This bacteria is a gram positive coccus, like Staphylococcus and Micrococcus. It belongs to the phylum Actinobacteria, class Actinobacteria, order Actinomycetalis, suborder Micrococcinae and family Micrococcaceae. *Kocuria* comprises of 18 species based on 16SrRNA phylogenetic studies, out of which 5 are supposed to be pathogenic for humans – *Kocuria varians*, *Kocuria kristinae*, *Kocuria rosea*, *Kocuria rhizophila*, *Kocuria marina*.[1,2]

It is a hard task to identify the bacteria. They are gram-positive cocci and are catalase positive but coagulase negative, thus often causing confusion with coagulase negative staphylococci (CoNS). They appear in tetrads like Micrococcus, although sometimes in larger clusters as well. One important feature is they tend to produce non-diffusible yellow colour on blood agar only when incubated for more than 48 hours. They show a good deal of variation in biochemical reactions. However, unlike Staphylococci, they are susceptible to bacitracin and lysozyme and resistant to furazolidone, nitrofurantoin and lysostaphin. They are modified oxidase test negative, and thus are different from Micrococcus.[3] All these morphological and biochemical parameters are important in this respect that in the Vitek ID-GPC-card panel test, *Kocuria* isolates are positive for only the alanine aryl amidase reaction and alkalinization of L-lactate.[1] Although recent studies show correct identification of *Kocuria* using the Vitek-2 ID-GPC gram-positive identification card, perhaps due to the recently introduced larger database that allows the identification of additional taxa, still morphological and biochemical parameters are very much useful to aid in diagnosis.[4,5]

Here we present some cases in the pediatric age group caused by *Kocuria spp.*, diagnosed with the aid of morphological and biochemical parameters followed by Vitek 2 confirmation. As there are no CLSI guidelines for antibiotic sensitivity testing for this emerging pathogen, we have performed Kirby-Bauer disc diffusion method and microbroth dilution following CLSI guidelines prescribed for Staphylococci, and have tried to outline a preliminary or basic sensitivity pattern for this bacteria.

MATERIALS AND METHODS

The study was done over a period of 7 months. Samples from which *Kocuria* spp. were isolated comprised mainly of blood culture, followed by pus, central venous catheter (CVC) tip and endotracheal tube (ET). Blood cultures were done on BactAlert 3D, followed by subculture on sheep Blood agar. Other samples were directly cultured on Blood agar. On Blood agar, they showed 2-3 mm., whitish, round, convex colonies without any hemolysis, and on or around 48 hrs of incubation showed non-diffusible yellow pigmentation. Under microscope, darkly stained large-sized cocci were found, arranged in pairs, tetrads, clusters and short chains. They were catalase positive and coagulase negative. Subsequent additional tests, such as bacitracin susceptibility, lysozyme sensitivity, resistance to furazolidone and lysostaphin were done followed by Modified oxidase test which stood negative.

These morphological and biochemical features suggestive of *Kocuria* were confirmed by Vitek-2 system (bioMe´rieux) of 64 tests; the time period spanned from 5 – 8.25 hours. Susceptibility testing by modified Kirby-Bauer disc diffusion technique and minimum inhibitory concentration (MIC) by microbroth dilution method was performed according to the Clinical and Laboratory Standards Institute guidelines for Staphylococcus. [CLSI 2018] All the patients were from pediatric age group, including infants and neonates.

RESULTS

There were four *Kocuria spp.* causing infections – *K. kristinae*, *K. rosea*, *K. varians* and *K. rhizophila*. All these four variants caused bloodstream infections (22 blood culture samples). *K. kristinae* and *K. rosea* were found in pus also. CSF infection was caused by *K. rosea*. The antibiotic susceptibility profiles of the four *Kocuria* spp. are included in tables 1 and 2.

TABLE – 1 Antibiotic susceptibility profiles of *Kocuria* spp. from samples other than blood.

SAMPLE	AGE	*Kocuria* spp	SUSCEPTIBLE TO	RESISTANT TO
Pus	2m	*Kocuria kristinae*	Amoxy-clav, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Cefotaxime, TMP-SMX
Pus	3y6m	*Kocuria rosea*	Linezolid, Tigecycline, Vancomycin	Amoxy-clav, TMP-SMX, Cefotaxime,Cefuroxime, Pip-Tazo, Meropenem,

Pus	18d	*Kocuria kristinae*	Amoxy-clav, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Cefotaxime, TMP-SMX
CSF	6m	*Kocuria rosea*	Cefotaxime,Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Amoxy-clav, TMP-SMX
ET Tube	15d	*Kocuria kristinae*	Vancomycin	Amoxy-clav, Cefuroxime, Cefotaxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Gentamycin
Central venous catheter	8d	*Kocuria kristinae*	Amoxy-clav, Cefuroxime, Cefotaxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline,	TMP-SMX, Gentamycin

TABLE 2: Kocuria spp in blood culture samples and their susceptibility patterns.

AGE	*Kocuria spp.*	SUSCEPTIBLE TO	RESISTANT TO
2m	*Kocuria rosea*	Linezolid, Vancomycin	Amoxy-clav, TMP-SMX,Cefotaxime,Cefuroxime, Pip-Tazo, Meropenem, Tigecycline,
1d	*Kocuria kristinae*	Cefotaxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Cefuroxime, TMP-SMX
1d	*Kocuria rhizophila*	Pip-Tazo, Tigecycline	Amoxy-clav, TMP-SMX,Cefotaxime,Cefuroxime, Meropenem,
6d	*Kocuria kristinae*	Amoxy-clav, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Cefotaxime,Cefuroxime, TMP-SMX
3m	*Kocuria rosea*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	TMP-SMX
1m24 d	*Kocuria kristinae*	Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Amoxy-clav, Cefotaxime, TMP-SMX, Gentamycin
15d	*Kocuria rosea*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	TMP-SMX, Gentamycin
1d	*Kocuria rosea*	Linezolid, Tigecycline, Gentamycin, Vancomycin	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Meropenem, TMP-SMX
28d	*Kocuria varians*	Amoxy-clav, Pip-Tazo, Linezolid, Tigecycline, Vancomycin	Cefotaxime, Cefuroxime, Meropenem, TMP-SMX
29d	*Kocuria rosea*	Linezolid, Vancomycin	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Meropenem, Tigecycline, TMP-SMX
2d	*Kocuria varians*	Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin	Amoxy-clav,TMP-SMX, Gentamycin
4y	*Kocuria rosea*	Pip-Tazo, Meropenem, Tigecycline, Vancomycin	Amoxy-clav, Cefotaxime, Cefuroxime, Linezolid, TMP-SMX, Amikacin, Gentamycin
9m	*Kocuria kristinae*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Meropenem, Tigecycline, Vancomycin,	TMP-SMX
3d	*Kocuria kristinae*	Cefotaxime, Cefuroxime, Tigecycline, Linezolid, Vancomycin	Amoxy-clav, Pip-Tazo, Meropenem, TMP-SMX
4d	*Kocuria rosea*	Pip-Tazo, Tigecycline, Vancomycin	Amoxy-clav, Meropenem, Cefotaxime, Cefuroxime, Linezolid, TMP-SMX
3m	*Kocuria kristinae*	Gentamycin, Meropenem, Tigecycline, Linezolid, Vancomycin	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, TMP-SMX
3d	*Kocuria kristinae*	Cefuroxime, Pip-Tazo, Gentamycin, Meropenem, Tigecycline, Linezolid, Vancomycin	Amoxy-clav, Cefotaxime, TMP-SMX
7m	*Kocuria varians*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Gentamycin, Meropenem, Tigecycline, Linezolid, Vancomycin	TMP-SMX
15d	*Kocuria varians*	Amoxy-clav, Tigecycline, Linezolid, Vancomycin	Cefuroxime, Pip-Tazo, Gentamycin, Meropenem, TMP-SMX
3d	*Kocuria kristinae*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Gentamycin, Meropenem, Tigecycline, Linezolid, Vancomycin	TMP-SMX
27d	*Kocuria kristinae*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Gentamycin, Meropenem, Tigecycline, Vancomycin	TMP-SMX
7m	*Kocuria kristinae*	Amoxy-clav, Cefotaxime, Cefuroxime, Pip-Tazo, Linezolid, Gentamycin, Meropenem, Tigecycline, Vancomycin	TMP-SMX

DISCUSSION

Among Kocuria, *K. kristinae* was the first species to be described in 1974. It is known to cause catheter-related bacteremia and infective endocarditis. These infections have been associated with cancers, acute cholecystitis and other metabolic disorders.[6,7,8,9] A brain abscess caused by *Kocuria varians* has been described as causing a brain abscess in the right occipital region.[10] *K. marina* and *K. rhizophila* have also joined the emerging spectrum of *Kocuria* species causing human infections.[2,11] Cases of *K. marina* peritonitis are known, especially in patients undergoing CAPD. *K. rosea* infection was first described in a patient who had relapsed Hodgkin disease undergoing peripheral blood stem cell transplantation and presented with multiple episodes of febrile neutropenia.[12]Till 2013, only 10 cases of *Kocuria spp*. infections in pediatric age group were reported, three among them being published separately (pediatric ages being 4 months, 2 yrs, 3 yrs,); and a pediatric case series described 7 cases of *Kocuria spp.* infection.[5,13] In 2014 one case report of *Kocuria marina* in a 2-year-old boy has been reported.[14] In 2015 a case report came up presenting *Kocuria rosea* causing infective endocarditis in an immunocompetent child.[15] In 2016 two case reports have been presented, both being

central line infections.[16]

We present here a series of 28 pediatric cases. We found four *Kocuria spp.* causing infections – *K. kristinae*, *K. rosea*, *K. varians* and *K. rhizophila*. All the four variants caused bloodstream infections. *K. kristinae* and *K. rosea* were found in pus also. CSF infection was caused by *K. rosea*. CSF infection by *Kocuria rosea* has been reported earlier.

Insufficient literature is available to establish antibiotic susceptibility profile for this bacteria. However, available data show it to be broadly susceptible to most of the antibiotics. In general, isolated mentions of intermediate susceptibility to Ceftazidime and Cefotaxime are there.[17] Similarly, resistance of *K. varians* to Norfloxacin only has been reported.[18] *K. rhizophila* has been reported to be resistant to Ciprofloxacin and Erythromycin in one study.[19] Lee et al. has shown *K. marina* to be resistant to Tetracycline, and *K. kristinae* to Oxacillin and Cefazolin. We have found, however, some significant deviations from the above reports in the sensitivity patterns. Our main observations are

1. Out of 28 Kocuria isolates, 13 were multidrug resistant (MDR).
2. All the four *Kocuria spp.* in our study were uniformly resistant to Cotrimoxazole (TMP-SMX).
3. Susceptibility to Co-Amoxyclav was also poor (14 out of 28 cases), as was seen in *K. kristinae*, *K. rhizophila*, and *K. rosea*. The two isolates of *K. varians* were found susceptible.

CONCLUSIONS

Insufficient literatures are available regarding the pathogenicity spectrum of this upcoming pathogen. High clinical and microbiological correlations are needed to avoid ignoring this masquerading pathogen as a commensal. Hopefully the antimicrobial susceptibility patterns presented here might provide a clue for treatment till the CLSI guidelines are formed

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