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Review

Drug sensitivity and clinical impact of members of the genus *Kocuria*

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Organisms in the genus *Kocuria* are Gram-positive, coagulase-negative, coccoid actinobacteria belonging to the family *Micrococcaceae*, suborder *Micrococcineae*, order *Actinomycetales*. Sporadic reports in the literature have dealt with infections by *Kocuria* species, mostly in compromised hosts with serious underlying conditions. Nonetheless, the number of infectious processes caused by such bacteria may be higher than currently believed, given that misidentification by phenotypic assays has presumably affected estimates of the prevalence over the years. As a further cause for concern, guidelines for therapy of illnesses involving *Kocuria* species are lacking, mostly due to the absence of established criteria for evaluating *Kocuria* replication or growth inhibition in the presence of antibiotics. Therefore, breakpoints for staphylococci have been widely used throughout the literature to try to understand this pathogen's behaviour under drug exposure; unfortunately, this has sometimes created confusion, thus higlighting the urgent need for specific interpretive criteria, along with a deeper investigation into the resistance determinants within this genus. We therefore review the published data on cultural, genotypic and clinical aspects of the genus *Kocuria*, aiming to shed some light on these emerging nosocomial pathogens.

Introduction

The genus *Kocuria* was named after Miroslav Kocur, a Slovakian microbiologist, and belongs to the family *Micrococcaceae*, suborder *Micrococcineae*, order *Actinomycetales*, class *Actinobacteria* (Takarada *et al.*, 2008; Zhou *et al.*, 2008; Lee *et al.*, 2009; Stackebrandt *et al.*, 1995). It includes Gram-positive, strictly aerobic (a few exceptions are *Kocuria kristinae*, which is facultatively anaerobic, *Kocuria marina*, which may grow in 5 % CO_2, and *Kocuria rhizophila* strain DC2201, which can proliferate anaerobically), catalase-positive, coagulase-negative, non-haemolytic cocci. These are also non-encapsulated, non-endospore-forming, non-halophilic, mesophilic, non-motile and Voges–Proskauer (production of indole and acetoin)-negative, and do not possess mycolic or teichoic acids. *Kocuria* species can be differentiated from other members of the *Actinomycetales* based on the presence of galactosamine and glucosamine as main cell wall amino sugars, the peptidoglycan type L-Lys-$Ala_{3/4}$, the fatty acid anteisio-$C_{15:0}$, the polar lipids diphosphatidylglycerol and phosphatidylglycerol, MK-7(H_2) and MK-8(H_2) as major menaquinones and a DNA G+C content of 60.0–75.3 mol%, depending on the species. Organisms in the genus are environmental bacteria, as well as human skin and oropharynx mucosa commensals; nevertheless, they can be responsible for infectious processes which mostly complicate severe underlying diseases. Owing to misidentification by phenotypic typing over the years, clinical syndromes caused by these agents are believed to be rare; however, the prevalence of such infectious pathologies is presumably higher and will surely increase in the coming years, as soon as genome-based identification is available in clinical laboratories. Accordingly, drug resistances and the clinical impact of the genus have been poorly discussed in the literature so far; therefore, the present review tries to shed light on this emerging field in medical microbiology (Takarada *et al.*, 2008; Zhou *et al.*, 2008; Lee *et al.*, 2009; Li *et al.*, 2006; Kim *et al.*, 2004; Ma *et al.*, 2005; Boudewijns *et al.*, 2005; Stackebrandt *et al.*, 1995; Reddy *et al.*, 2003; Schleifer & Kandler, 1972; Collins *et al.*, 1977; Dunphy *et al.*, 1971; Rosenthal & Dziarski, 1994; Bohácek *et al.*, 1969; Ogasawara-Fujita & Sakaguchi, 1976; Staneck & Roberts, 1974; Becker *et al.*, 2008; Kovács *et al.*, 1999; Park *et al.*, 2010a, b; Tang *et al.*, 2009; Yun *et al.*, 2010; Seo *et al.*, 2009).

Taxonomy

The genus *Kocuria* was created from the genus *Micrococcus*, the members of which have been indicated over the years by the vernacular name 'micrococci'. Based on phylogenetic and

chemotaxonomic analysis, the genus *Micrococcus* was dissected by Stackebrandt *et al.* (1995) into the genera *Kocuria*, *Micrococcus*, *Kytococcus*, *Nesterenkonia* and *Dermacoccus*. These were then rearranged into the two families *Micrococcaceae* and *Dermacoccaceae*, both belonging to the suborder *Micrococcineae*. Currently, *Kocuria* is in the order *Actinomycetales*, class *Actinobacteria* (Takarada *et al.*, 2008; Stackebrandt *et al.*, 1995; Basaglia *et al.*, 2002; Becker *et al.*, 2008; Rainey *et al.*, 1996, 1997; Jagannadham *et al.*, 1991).

Seventeen *Kocuria* species have been so far described: *K. varians* (Stackebrandt *et al.*, 1995), *K. rosea* (formerly *Sarcina erythromyxa*, then *Micrococcus roseus*, *Deinococcus erythromyxa* and *K. erythromyxa*) (Stackebrandt *et al.*, 1995; Kovács *et al.*, 1999; Cooney & Berry, 1981; Schwartzel & Cooney, 1970, 1972; Ungers & Cooney, 1968; Thierry & Cooney, 1966), *K. kristinae* (Stackebrandt *et al.*, 1995), *K. palustris* (Kovács *et al.*, 1999), *K. rhizophila* (Kovács *et al.*, 1999), *K. marina* (Kim *et al.*, 2004), *K. polaris* (Reddy *et al.*, 2003), *K. aegyptia* (Li *et al.*, 2006), *K. carniphila* (Tvrzová *et al.*, 2005), *K. himachalensis* (Mayilraj *et al.*, 2006), *K. flava* (Zhou *et al.*, 2008), *K. turfanensis* (Zhou *et al.*, 2008), *K. atrinae* (Park *et al.*, 2010a), *K. gwangalliensis* (Seo *et al.*, 2009), *K. halotolerans* (Tang *et al.*, 2009), *K. koreensis* (Park *et al.*, 2010b) and *K. salsicia* (Yun *et al.*, 2010).

Isolation methods

Kocuria species usually grow on simple media (such as sheep blood agar); under a microscope, pairs, tetrads, clusters, packets or single cells may be observed. On the agar surface, colonies appear as orange, pink, red, yellow or cream, depending on the individual species and strain. Salient microscopic and cultural aspects are summarized in Table 1 (Ben-Ami *et al.*, 2005; Reddy *et al.*, 2003; Stackebrandt *et al.*, 1995; Mayilraj *et al.*, 2006; Li *et al.*, 2006; Kim *et al.*, 2004; Kovács *et al.*, 1999; Ma *et al.*, 2005; Boudewijns *et al.*, 2005; Zhou *et al.*, 2008; Lee *et al.*, 2009; Tvrzová *et al.*, 2005; Becker *et al.*, 2008; Takarada *et al.*, 2008; Park *et al.*, 2010a, b; Tang *et al.*, 2009; Yun *et al.*, 2010; Seo *et al.*, 2009).

Identification

Phenotype-based identification assays may fail to recognize *Kocuria* species. Biochemical features may in fact widely vary between members of the genus, and commercially available phenotype databases do not include all of the classified species. In particular, oxidase, amylase, urease, gelatinase, phosphatase and β-galactosidase activities, as well as carbon source (α-cyclodextrin, L-arabinose, D-gluconic acid, pyruvic acid methyl ester, methyl β-D-glucoside, *N*-acetyl-L-glutamic acid, α-ketoglutaric acid, α-D-lactose, inulin, *p*-hydroxyphenylacetic acid) utilization and nitrate reduction, are heterogeneously expressed in the different strains. Also, the DNA G+C content is known to range from 60.0 to 75.3 mol% among the 17 species described (Ben-Ami *et al.*, 2005; Boudewijns *et al.*, 2005; Lee *et al.*, 2009; Zhou *et al.*, 2008; Park *et al.*, 2010a, b; Tang *et al.*, 2009; Yun *et al.*, 2010; Seo *et al.*, 2009).

Correct identifications, as well as misidentifications, of *Kocuria* species by Vitek (bioMérieux), Vitek 2 (bioMérieux), API

Table 1. Salient microscopic and macroscopic aspects of *Kocuria* species, as reported in the literature

Species	Cell diameter and arrangement	Characteristics of colonies	Growth temperature
K. rosea	1–1.5 μm; pairs, tetrads and clusters	Smooth or rough, slightly convex; orange, red or pink	25–37 °C
K. varians	0.9–1.5 μm; single cells, tetrads, irregular clusters of tetrads, packets	Slightly convex; smooth and glistening, or rough, matt, wrinkled and dry; yellow	22–37 °C
K. kristinae	0.7–1.1 μm; tetrads, clusters of tetrads	Crenate or entire, smooth or rough, convex; pale-cream to pale-orange	25–37 °C
K. flava	–	Yellow	28–45 °C
K. turfanensis	–	Yellow to orange	30–40 °C
K. carniphila	1.5 μm; pairs and tetrads	Convex; opaque; yellow to orange	28–37 °C
K. marina	Pairs, tetrads, clusters	Yellow to orange	4–43 °C
K. salsicia	1.0–1.5 μm	Circular with entire margins; opaque; lemon-yellow	15–37 °C
K. halotolerans	0.6–1.0 μm	Circular, slightly convex, smooth; opaque; pale-yellow	10–37°C
K. atrinae	1.0–1.5 μm	Circular, smooth; opaque; pale-yellow	25–37°C
K. gwangalliensis	0.6–1.2 μm; pairs	Smooth, convex; pink to orange	10–37°C
K. koreensis	1.0–1.5 μm	Circular, smooth; opaque; pale-cream to pale-orange	15–37 °C
K. palustris	1.0–1.5 μm (rarely 2.0 μm); pairs, tetrads, packets	Smooth, with irregular edges; pale-yellow	>5–30 °C (strain TAGA27^T: 10–30 °C)
K. rhizophila	1.0–1.5 μm (rarely 2.0 μm); pairs, tetrads, packets	Smooth, with irregular edges, creamy; opaque; yellow to orange	10–40 °C
K. himachalensis	1.0–1.5 μm; pairs, tetrads, clusters	Reddish-orange	20–37 °C
K. polaris	1.0–1.5 μm; pairs, tetrads, clusters	Smooth, mucoid, uniformly edged; translucent; yellow to orange	5–30 °C (strain MTCC 3702: 5–42 °C)
K. aegyptia	0.8–1.1 μm; pairs, tetrads, clusters	Slightly convex; opaque; pink	20–40 °C

(bioMérieux) and the BD Phoenix identification systems are summarized in Table 2 (Lee *et al.*, 2009; Becker *et al.*, 2008; Ma *et al.*, 2005; Boudewijns *et al.*, 2005; Basaglia *et al.*, 2002; Lai *et al.*, 2010).

Although it is clear that genome-based typing is needed to obtain exact characterization of *Kocuria* species, a preliminary identification may be provided by phenotypic tests. In particular, major criteria for the conventional discrimination between micrococci and staphylococci are the sensitivity of *Kocuria* to bacitracin and lysozyme (while staphylococci are resistant to both) and the resistance of *Kocuria* to nitrofurantoin/furazolidone and lysostaphin (staphylococci are susceptible to the latter, although they may express resistance to the former) (Becker *et al.*, 2008; Boudewijns *et al.*, 2005; Mashouf *et al.*, 2009).

Disease

Infectious pathologies caused by *Kocuria* species are believed to be unusual, so they have been poorly studied over the past years (Table 3). *Kocuria* appears to mostly affect compromised hosts suffering from haematological malignancies, solid tumours or metabolic disorders, although only *K. kristinae*, *K. marina* and *K. rhizophila* have been observed to cause infections in humans, to the best of our knowledge (Lee *et al.*, 2009). Furthermore, the peritonitis and bacteraemia episodes described by Kaya *et al.* (2009) and Altuntas *et al.* (2004), respectively, were presumably caused by *K. rosea*, but genome-based confirmation was not provided in either case. Therefore, attribution of the clinical pictures described by the authors to *K. rosea* or *Kocuria* species may be debatable.

Epidemiology

Kocuria species are skin and oropharynx commensals in mammals (including man), as well as environmental organisms inhabiting the soil and several other ecological niches (Altuntas *et al.*, 2004; Becker *et al.*, 2008; Lee *et al.*, 2009; Zhou *et al.*, 2008; Schumann *et al.*, 2000). *K. aegyptia* was isolated from a saline, alkaline desert-soil sample from Egypt (Li *et al.*, 2006); *K. marina* was found to inhabit marine sediment in the East Siberian Sea (Kim *et al.*, 2004); *K. carniphila* was observed to colonize meat (Tvrzová *et al.*, 2005); *K. polaris* was collected from a cyanobacterial mat sample originating from Antarctica (Reddy *et al.*, 2003); *K. rhizophila* and *K. palustris* were cultured from the rhizoplane of the narrow-leaved cattail (*Typha angustifolia*), which inhabits the Danube River (Kovács *et al.*, 1999). Further, the former has been recognized as the predominant bacterium in chicken meat treated with oxalic acid (the latter is used to reduce the count of naturally colonizing organisms on raw chicken). Becker *et al.* (2008) also suggested that *K. rhizophila* contaminated dust, freshwater or food, although the authors did not provide any confirmation of such a hypothesis. Both *K. koreensis* and *K. atrinae* were isolated in Korea from jeotgal, a traditional, fermented seafood made from comb pen shell (Park *et al.*, 2010a, b). *K. salsicia* was also cultured in Korea from a salt-fermented food (flatfish) called 'gajami-sikhae' (Yun *et al.*, 2010). *K. gwangalliensis* has also been described in Korea as a marine bacterium inhabiting the Gwangalli coast (Seo *et al.*, 2009), whereas *K. halotolerans* was collected from a saline soil sample from a forest reserve in China (Tang *et al.*, 2009). Finally, *K. flava* and *K. turfanensis* were recovered as airborne organisms from Xinjiang, China (Zhou *et al.*, 2008), while *K. himachalensis* was collected from soil in a cold desert of the Indian Himalayas, below an ice glacier, 4200 m above sea level (Mayilraj *et al.*, 2006).

Antibiotic resistance

Apart from nitrofurantoin/furazolidone resistance, which has been included among the major criteria for preliminary phenotypic identification of *Kocuria* species (see the section Identification), drug resistance exerted by these organisms has been poorly investigated over the decades. Sensitivities reported in the literature are described in Table 4. Of interest, only kanamycin resistance has been documented among the aminoglycosides, while variable susceptibilities to β-lactams, quinolones, lincosamides and cotrimoxazole have been observed. Notably, no case of resistance to glycopeptides, streptogramins, fusidic acid, rifampicin or linezolid has been reported so far, while polymyxin susceptibility, although described, is unexpected, and has prudently not been reported in the table; Gram-positive bacteria are in fact known to inherently express resistance to colistin and polymyxins in general, so we feel that the sensitivity of *Kocuria* is unusual (Savini *et al.*, 2009b). Again,

Table 2. Identification and misidentification of *Kocuria* species by phenotype-based systems, as found in the literature

Molecular ID*	Vitek 2	Vitek	API	Phoenix
K. marina	*K. varians*, *K. kristinae*	–	*K. kristinae*, *Staphylococcus chromogenes*	*K. varians*
K. rhizophila	*K. varians*, *K. rosea*, *Dermacoccus nishinomiyaensis*, *Micrococcus luteus*	*Staphylococcus auricularis*, *Staphylococcus capitis*	–	–
K. kristinae	*K. kristinae*	–	*K. kristinae*	*K. kristinae*

*ID, Identification.

Table 3. *Kocuria* species-associated diseases and comorbidities/risk factors discussed in the literature

Species	Disease	Comorbidity/risk factor	Reference
K. marina	Peritonitis	Continuous peritoneal dialysis	Lee *et al.* (2009)
K. rosea	Peritonitis*	Continuous peritoneal dialysis	Kaya *et al.* (2009)
	Catheter-related bacteraemia*	Hodgkin's disease under PBSC† transplantation	Altuntas *et al.* (2004)
K. rhizophila	Port-a-Cath-related bacteraemia	Methylmalonic aciduria	Becker *et al.* (2008)
K. kristinae	Acute cholecystitis	Post-laparoscopic cholecystectomy	Ma *et al.* (2005)
	Catheter/Port-a-Cath-related bacteraemia	Ovarian cancer	Basaglia *et al.* (2002)
		Gastric cancer	Lai *et al.* (2010)
		Congenital short bowel syndrome	
		Hypogammaglobulinaemia	
	Infective endocarditis	Ischaemic bowel disease, short bowel syndrome	Lai *et al.* (2010)

*Presumed identification (phenotypic characterization).
†Peripheral blood stem cells.

Stackebrandt *et al.* (1995) state that 'most' *K. varians* strains show tetracycline, chloramphenicol, erythromycin, oleandomycin, streptomycin and penicillin G sensitivity, so we understand that a few isolates may exert resistance to these drugs. Such resistances have not been included in the table as they can only be presumed. Also, Becker *et al.* (2008) found that *K. rhizophila* exerted β-lactam, macrolide, glycopeptide and quinolone sensitivity (with the exception of norfloxacin resistance) if the operator of the Vitek 2 system declared the isolates as coagulase-negative staphylococci (CoNS). The authors did not specify which molecules within the antibiotic classes cited were tested, however (Stackebrandt *et al.*, 1995; Basaglia *et al.*, 2002; Zhou *et al.*, 2008; Lee *et al.*, 2009; Reddy *et al.*, 2003; Kim *et al.*, 2004; Kovács *et al.*, 1999; Ma *et al.*, 2005; Lai *et al.*, 2010; Becker *et al.*, 2008).

Table 4 does not include the sensitivities reported by Szczerba (2003). The latter publication is in Polish, except for the abstract, which is in English. In this section, the author labels most strains as resistant to ampicillin and erythromycin, but susceptible to doxycycline, amoxicillin/clavulanate, ceftriaxone, cefuroxime, ciprofloxacin, cotrimoxazole and amikacin. Unfortunately, these data globally refer to the genera *Kocuria*, *Micrococcus*, *Nesterenkonia*, *Kytococcus* and *Dermacoccus* taken together, while no precise individual information is reported. Also, we did not report in the table the MICs observed by Lai *et al.* (2010), which did not clearly belong to any of the sensitive or resistant categories (according to interpretive criteria for staphylococci).

Of interest, Altuntas *et al.* (2004) observed that a *K. rosea* strain from a catheter-related bacteraemia expressed vancomycin sensitivity *in vitro*; however, drug administration did not change the clinical picture of the infectious episode until the catheter was removed. It was then hypothesized that the formation of biofilm on the device surface was able to protect the bacterial communities from the antibiotic activity. Tip culture was performed after removal, from which *K. rosea* grew with high bacterial counts. No evidence of *Kocuria* biofilm production has been provided, however, in this article or elsewhere in the literature; hence, this just remains a hypothesis (Altuntas *et al.*, 2004; Savini *et al.*, 2010).

Very limited data are cited throughout the literature concerning antibiotic resistance mechanisms in *Kocuria* species. Takarada *et al.* (2008) reported that the *K. rhizophila* strain DC2201 genome contains 13 proteins probably involved in a multidrug efflux mechanism. The latter catalyses the active extrusion of several structurally and functionally unrelated molecules from the cytoplasm to the external medium, and presumably transports toxic organic compounds outward (including solvents to which the strain is notoriously tolerant). Of interest, one of the *K. rhizophila* efflux proteins has homology with the plant pathogen *Xanthomonas albilineans* pump, thus suggesting the association of strain DC2201 with plant environments, where it shares ecological niches with plant pathogens. Unfortunately, it is still unclear to what extent efflux activities affect drug sensitivities, as well as to what extent further resistance mechanisms may determine antibiotic resistance within *Kocuria* species (Takarada *et al.*, 2008).

Concluding remarks

Micrococci have been hastily labelled as simple contaminants in past decades, apart from strains (i.e. *K. rhizophila* ATCC 9341) employed as quality controls in industrial applications, including antibiotic sensitivity testing (Takarada *et al.*, 2008; Tang & Gillevet, 2003). Currently, these bacteria are gaining importance as emerging pathogens in hosts with cancer, immunocompromised status and metabolic disorders. In particular, their affinity for plastic materials is worrying; infected devices may in fact cause chronic recurrent bacteraemias and represent a cause of morbidity and mortality in compromised hosts. *Staphylococcus aureus*, CoNS, Gram-negative bacilli and *Candida albicans* are the major agents of device-related septicaemia, *Kocuria* species only recently being recognized as catheter-infecting pathogens (although no biofilm

Table 4. *In vitro* sensitivities of *Kocuria* species to antibiotics according to the literature

Entries in parentheses indicate the absence of specified methods for sensitivity testing. S, Susceptibility; **R**, resistance; S, moderate sensitivity.

Antibiotic	*K. varians*	*K. kristinae*	*K. rosea*	*K. flava*/ *K. turfanensis*	*K. marina*	*K. polaris*	*K. rhizophila*	*K. palustris*
Gentamicin					S*			
Amikacin						(S)		
Tobramycin						(S)		
Netilmycin	S†	S†	S†				S†	S†
Kanamycin	(S)	(S)	(S)	(S)	(**R**)	(S)		
Neomycin	(S)	(S)	(S)		(S)			
Streptomycin	(S)		(S)		(S)	(S)		
Non-specified polymyxin/ polymyxin B	(S)	(S)	(S)		(**R**)			
Colistin (polymyxin E)						(**R**)		
Vancomycin	S†	S*†‡§	S†		S*‡	(S)	S†	S†
Teicoplanin		S‡			S‡			
Penicillin G/benzylpenicillin	S†	S*†‡	S†		S*/**R**‡	(S)	S†	S†
Ampicillin/amoxicillin	S†	S†‡	S†	(S)	S*‡	(S)	S†	S†
Ampicillin–sulbactam	S†	S†	S†		S*		S†	S†
Amoxicillin–clavulanate		S‡			S‡			
Meticillin/oxacillin	(S)	S†§/**R**‡	(S)		S‡			
Cloxacillin		S*						
Carbenicillin				(S)	(S)			
Piperacillin	S†	S†	S†				S†	S†
Cefalexin	S†	S†	S†				S†	S†
Cefamandol	S†	S†	S†				S†	S†
Cephalotin		S†§			S*			
Cefuroxime						(S)		
Cefoperazone	S†	S†	S†			(S)	S†	S†
Cephazolin						(S)		
Cefotaxime						(S)		
Meropenem	S†	S†	S†				S†	S†
Tetracycline	(S)	(S)	(S)		S*	(S)		
Chlortetracycline				(S)				
Chloramphenicol	S†	S†	S†		S*	(S)	S†	S†
Quinupristin–dalfopristin		S‡			S‡			
Fusidic acid		S‡			S‡			
Erythromycin	S†	S*†§‡	S†	(S)	S*‡	(S)	S†	S†
Roxithromycin						(S)		
Oleandomycin	(S)		(S)		(S)			
Clindamycin	S†	S*†‡§/**R**‡	S†		S*‡		S†	S†
Lincomycin					(S)	(S)		
Ciprofloxacin		S†‡§			S*‡	(S)	**R**†	S†
Levofloxacin		S*‡			S‡			
Moxifloxacin					S*			
Lomefloxacin						(S)		
Norfloxacin						(S)	**R**†	
Nalidixic acid						(**R**)		
Rifampicin					S*			
Cotrimoxazole		S*			S*/**R***	(S)		
Trimethoprim	S†	S†	S†				S†	S†
Linezolid		S*‡			S‡			

*NCCLS/CLSI agar method (interpretive criteria for staphylococci).
†NCCLS/CLSI agar method (no interpretive criteria specified).
‡BD Phoenix (interpretive criteria for staphylococci).
§bioMérieux Vitek/Vitek 2 (no interpretive criteria specified).

production has been demonstrated so far, as we said before). We therefore suggest that physicians should not underestimate the importance of such micro-organisms when isolated from clinical samples, especially blood and inert medical implant surfaces (Altuntas *et al.*, 2004; Lee *et al.*, 2009; Basaglia *et al.*, 2002).

Most data in the literature refer to micrococci in general, so the clinical significance of each individual genus has been poorly discussed over the years. *Kocuria* is difficult to characterize by phenotypic methodologies, as commercially available databases do not include all of the classified species. Furthermore, both micrococci and staphylococci are known to show phenotypic variability, which may lead to failure of biochemical identification. In particular, changes in colony morphology, exopolysaccharide production, capacity to adhere to inert materials and expression of drug resistance are typical virulence traits in *Staphylococcus epidermidis* pathogenic strains, whereas commensal skin isolates do not show any modification. Also, the ability to vary is presumed to enhance microbial survival and growth under modifying environmental conditions. Varying staphylococcal and micrococcal strains may be misidentified by phenotype-based assays, as biochemical activities can change during replication on agar media in order to allow microbes to adapt to changing environmental conditions (Ben-Ami *et al.*, 2003, 2005; Lee *et al.*, 2009; Savini *et al.*, 2009a). We further highlight that genome analysis by 16S rRNA sequencing is needed to provide correct characterization at the genus and species level, whereas PFGE may be useful in confirming the clonality of different isolates. For instance, in the work of Altuntas *et al.* (2004), no genome comparison was performed, so it could only be supposed that the blood and catheter *K. rosea* isolates actually were the same strain. The episode resolved after device removal, however, so the authors considered the infectious process a catheter-related bacteraemia. High cost and the intensive labour required by molecular typing may unfortunately limit the use of such methodologies in routine practice, so a number of *Kocuria* species are presumed to be misidentified daily in clinical laboratories (Altuntas *et al.*, 2004; Basaglia *et al.*, 2002; Lai *et al.*, 2010). In this context, we underline that this greatly affects epidemiological investigations, studies on drug resistances and understanding of the saprophytic or pathogenic role of *Kocuria* clinical isolates, since these could be wrongly labelled as Gram-positive cocci other than *Kocuria*, or simply and hastily classified as micrococci.

As well as *Kocuria* species being misidentified by phenotypic tests, misidentification of CoNS (*S. epidermidis*/*Staphylococcus haemolyticus*, of which 58 % are phenotypically variable) as *Kocuria* species (*K. varians*/*K. rosea*) using the Vitek 2 system has been reported in the literature. Even in this case, therefore, 16S rRNA gene sequencing has been evoked to eliminate confusion arising from biochemical variation of clinical strains. Furthermore, as the identification of CoNS isolates as *Kocuria* species by Vitek 2 is thought to be frequent, Ben-Ami proposed that they be simply reported as 'unidentified Gram-positive cocci' (Ma *et al.*, 2005; Boudewijns *et al.*, 2005; Ben-Ami *et al.*, 2003, 2005). Hence, in light of what we have discussed, we would like to suggest that bacitracin, lysozyme, lysostaphin and nitrofurantoin/furazolidone (see the section Identification) are always tested with the aim of providing a preliminary discrimination between the genera *Kocuria* and *Staphylococcus*. In particular, we feel that it may be easy and cost-effective to routinely incorporate bacitracin and nitrofurantoin/furazolidone tests in sensitivity assays with Gram-positive isolates that have been biochemically recognized as *Kocuria*. Again, it is known that the typical pigmentation of *Kocuria* colonies usually gets more distinct with age (Ben-Ami *et al.*, 2005; Reddy *et al.*, 2003; Stackebrandt *et al.*, 1995; Mayilraj *et al.*, 2006; Li *et al.*, 2006; Kim *et al.*, 2004; Kovács *et al.*, 1999; Ma *et al.*, 2005; Boudewijns *et al.*, 2005; Zhou *et al.*, 2008; Lee *et al.*, 2009; Tvrzová *et al.*, 2005; Becker *et al.*, 2008; Takarada *et al.*, 2008). Hence, when *Kocuria* is identified by phenotype-based systems, we think that it could be useful to prolong plate incubation for $\geqslant$48 h to better appreciate colony pigmentation and discriminate between staphylococcal and *Kocuria* isolates.

In light of all of the above reported considerations, we believe that the prevalence of human diseases caused by *Kocuria* species is currently underestimated. A number of presumed staphylococcal pathologies may have been caused by *Kocuria* species over the years; conversely, it is conceivable that a variety of presumed *Kocuria* infections were actually caused by CoNS.

Concerning antibiotic therapy, proper treatment for *Kocuria* infections has not yet been defined, although members of the genus so far show susceptibility to most anti-infective drugs. Szczerba (2003) proposed amoxicillin/clavulanate (along with ceftriaxone, cefuroxime, doxycycline and amikacin) as first-line therapy against micrococcal pathologies. In any case, treatment duration should depend on the infection site, and a 10–14 day period of administration is suggested when a bacteraemia is diagnosed. Finally, catheter removal is recommended when trying to treat device-related bloodstream infections, in which biofilm formation may be reasonably presumed to play a pathogenic role. Infusion of antibiotics (especially if showing antibiofilm activity) in a catheter lock could also be useful in managing an implant-related sepsis and trying to save the vascular access (Ma *et al.*, 2005; Szczerba, 2003; Lai *et al.*, 2010; Savini *et al.*, 2010).

We now consider the work of Szczerba (2003) (see the section Antibiotic resistance), in the abstract of which the author described most strains as ampicillin-resistant but amoxicillin/clavulanate-susceptible. Unexpectedly, no β-lactamase production was documented (Szczerba, 2003); hence, we wonder what kind of resistance mechanism in Gram-positive bacteria (other than β-lactamase production) could affect ampicillin but not amoxicillin/clavulanate. The article is in Polish (with the exception of the

abstract), however, so we could not find any clarification of this issue in the text. Penicillin-binding protein mutation (if any) would affect both ampicillin and the β-lactamase inhibitor (as occurs in meticillin-resistant staphylococci) so perhaps resistance involves the expression of cell wall impermeability or efflux pumps affecting ampicillin (alone) rather than amoxicillin/clavulanate. However, this remains an unanswered question to our knowledge. Lai *et al.* (2010) described four *K. kristinae* strains to be penicillin- and oxacillin-resistant (based on interpretive criteria for staphylococci), but ampicillin- and amoxicillin/clavulanate-susceptible. Hence, we wonder why the isolates express resistance to penicillin but not to ampicillin, if we assume that *Kocuria* resistance mechanisms are shared with staphylococci. Nonetheless, clavulanate sensitivity appears unexpected in an oxacillin-resistant strain; similarly, *K. marina* is presented as resistant to penicillin but susceptible to ampicillin. In this context, the authors underlined the lack of specific MIC and inhibition zone diameter (on agar media) breakpoints and ranges for *Kocuria* species; in the absence of these, sensitivities in the literature mostly refer to *Staphylococcus* interpretive values, with the risk of misdiagnosing either sensitivity or resistance cases. We therefore emphasize the need for specific criteria for interpreting sensitivity assays with *Kocuria* isolates and a deeper investigation into resistance mechanisms expressed in this genus.

In particular, we feel that oxacillin (meticillin) resistance may represent a matter of concern. Although described in *K. kristinae* (see Table 4), no explanation for it exists in the literature to our knowledge. We presume that, as in staphylococci, penicillin-binding protein mutation may confer resistance to oxacillin (meticillin). Therefore, while waiting for a deeper understanding of such an issue, we suggest the prudent use of the Clinical and Laboratory Standards Institute $\geqslant 0.5$ μg ml^{-1} breakpoint for CoNS oxacillin resistance (rather than that for *S. aureus*/*Staphylococcus lugdunensis*, which is $\geqslant 4$ μg ml^{-1}), in order not to underestimate resistance spread within the genus (CLSI, 2008). We also suggest that (as in staphylococci) all oxacillin-resistant isolates should be prudently considered as pan-β-lactam-resistant to avoid *in vivo* failure of penicillin, cephalosporin and carbapenem therapy.

To conclude, much is still unclear about *Kocuria* species, which are a poorly explored field of medical microbiology. Further genome-based investigation is needed to shed light on the environmental spread, hospital epidemiology and clinical impact of this pathogen; in particular, mechanisms of pathogenicity and drug resistance still have to be more fully understood. Also, to what extent resistance to antibiotics of this organism may be acquired after drug exposure, inherently expressed by members of the genus or transmitted by mobile DNA among different strains within each *Kocuria* species, among different species within the genus, or from and to bacterial organisms other than *Kocuria* should be investigated and clarified.

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