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Airborne Coagulase Negative Staphylococci in the Indoor Environment of King Abdullah University Hospital, Jordan, and their Antibiotic Susceptibility

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Abstract

This study aimed to determine the density of airborne coagulase negative staphylococci (CoNS) in the indoor environment of the operating theatres (OT), intensive care units (ICU) and nursery intensive care units (NICU) at King Abdullah University Hospital, Jordan, and to evaluate their susceptibility to different antibiotics. Different identified gram-positive bacteria were previously isolated from 34 air samples of the different wards studied. CoNS were the most prevalent of all gram positives in OT (1.65%), ICU (3.78%) and NICU (1.55%) with *Staphylococcus saprophyticus* being the most frequent (>65%) among the isolated species. CoNS were the most commonly isolated gram-positive cocci in ICU and showed a remarkable resistance to novobiocin but high susceptibility to ciprofloxacin.

Keywords*:* airborne; antibiotic; cons; hospital; *Staphylococci*

Резюме

Това проучване има за цел да определи плътността на въздушно-пренасяните коагулазо-отрицателни стафилококи (КоОС) във вътрешната среда на операционните зали (ОЗ), отделенията за интензивно лечение (ОИЛ) и детските отделения за интензивно лечение (ДОИЛ) в университетската болница King Abdullah, Йордания, а така също да се оцени тяхната чувствителност към различни антибиотици. По-рано бяха идентифицирани различни грам-положителни бактерии, изолирани от 34 въздушни проби от съответните отделения. КоОС са най-разпространени от всички грам положителни резултати в ОЗ (1.65%), ОИЛ (3.78%) и ДОИЛ (1.55%), като *Staphylococcus saprophyticus* е най-честият (>65%) сред изолираните видове. КоОС са най-често изолираните грам-положителни коки в интензивното отделение и показват забележителна резистентност към новобиоцин, но висока чувствителност към ципрофлоксацин.

Introduction

Indoor air quality of hospitals is remarkably important because the hospital environment is full of pathogens which may cause nosocomial and other infections through airborne exposure. Airborne bacteria are one of these pathogens and the evaluation of their count, types and diversity in hospital rooms, especially operating theaters (OT), intensive care units (ICU) and nursery intensive care units (NICU), is very important for the control and prevention of hospital-acquired infections (HAI).

Coagulase negative staphylococci (CoNS) are one of the major bacterial species present in the indoor hospital environment (Suzuki *et al.*, 1984) and thus may contaminate the environment of OT, ICU and others (Vlodavets *et al.*, 1980). CoNS comprises a large group of related gram-positive species, of which *Staphylococcus epidermidis* is the important

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one as it has become a common cause of surgical site infection. Methicillin resistant *S. epidermidis* (MRSE) strains have become a worldwide problem and studies have indicated that MRSE causes 15% of postoperative infections (Tammelin *et al.*, 2000). For this reason, the objective of this study was to measure the quantities of airborne CoNS bacteria in different units of King Abdullah University Hospital, Jordan. The susceptibility of CoNS to commonly used antibiotics was also evaluated.

The extensive use of some antibiotics in hospitals for longer periods may lead to higher resistance percentage of CoNS. Therefore, monitoring the presence and distribution of CoNS in the hospital environment should continue. In a previous study for the antibiotic susceptibility and concentration of airborne CoNS in the intensive care units of different hospitals in Northern Jordan, Saadoun and his collegues (Saadoun *et al*., 2014a) found that CoNS were the most prevalent bacteria of all Gram positives in ICU and NICU and showed remarkable resistance to Novobiocin but high susceptibility to Ciprofloxacin. However, antibiotic sensitivity of CoNS in the indoor environment of a referral university hospital, King Abdullah University Hospital (KAUH), Jordan, to commonly used antibiotics, has not been studied before.

Materials and Methods

The studied hospital

The King Abdullah University Hospital (KAUH) is a modern general hospital located in Jordan University of Science and Technology (JUST) in Irbid city in the north of Jordan. The hospital was established in 2002 with a total capacity of 850 beds and a total staff of over 2000, and has been used for monitoring purposes. Density of airborne Gram positive cocci bacteria were determined OT, ICU and NICU of the hospital. Each ICU and NICU consisted of three separated different size rooms and OT consisted of eight operating rooms. The temperature in these three rooms is maintained at 22°C±2°C all year around.

Collection of air samples and sample processing

A total of 34 air samples of 100 liters volume/min were previously collected by a microbiological air sampler (Oxoid, UK) from the different studied wards of the hospital, and then processed as described by Saadoun *et al*. (2104a, 2014b).

Isolation and identification of bacteria

Each bacterial colony recovered on agar plates was sub-cultured on trypticase soy agar (TSA) or blood agar with 5-7% defibrinated blood (Oxoid, UK) at 35°C for 24-48 h. Macroscopic and microscopic examination in addition to other specific biochemical tests were performed to identify each bacterial colony. A pure colony was picked up from each culture plate, then a smear was prepared on a clean microscopic glass slide, dried, fixed by heating and Gram stained according to the standard methods (Balows *et al.*, 1991). All slides were microscopically evaluated at 100 X. According to the results of Gram stain, each bacterial colony was sub-cultured under aseptic conditions on different culture media for isolation, identification, and testing the susceptibility of the isolates to common used antibiotics (Table 1).

Identification of CoNS

The identification process was based on the results of Gram staining. Catalase activity was the first step in identifying *Staphylococcus* spp., which was performed to the standard methods (Koneman *et al.*, 1997) and on the morphology of colonies (medium to large, smooth, entire, slightly raised, translucent), and showing clusters of gram positive. They can grow on mannitol salt agar (Oxoid, UK) and their coagulation of plasma indicates positive results of coagulase production, especially for *S. aureus*. All of the above tests were performed according to standard methods (Balows *et al.*, 1991).

Antimicrobial susceptibility test

Plates and inoculums preparation. Test organism from the isolated culture was activated by inoculation into Mueller-Hinton broth or trypticase soy broth (Oxoid, UK), then incubated at 37°C for 24 h. Turbidity of organisms in the broth was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5×10^8 cfu/ml). Mueller-Hinton agar plates and 0.5 McFarland standards were prepared according to the recommendation of Kirby-Bauer disc diffusion procedure (Bauer *et al.*, 1966) and National Committee for Clinical Laboratory (NCCLS, 2003). The test organisms were homogeneously inoculated by a sterile cotton swab on the surface of two freshly prepared Mueller-Hinton (Oxoid, UK) agar plates. Plates were incubated at 37°C for 24 h.

Application of disks and incubation

Twenty-three different antibiotics disc (Oxoid, UK) (Table 1) were used for susceptibility test of CoNS. Antibiotic disks were applied to the agar surface by sterile forceps and then gently pressed to assure complete contact of disks on

the agar surface. Plates were then inverted and incubated at 35°C for 16-18 h. The results of the susceptibility test (inhibition zone diameter) for each isolate were measured in (mm) and interpreted according to NCCLS standards (NCCLS, 2003) on a special data sheet.

Results and Discussion

Airborne bacteria are the major type of microorganisms present in all hospital environments (Beggs, 2003). *S. saprophyticus* (Novobiocin resistance) has been found to comprise 66.9% of all coagulase negative *Staphylococcus* while *Staphylococcus* spp. comprises the other 33.1% (data not shown). This result indicates that the major part of CoNS in the hospital air is related to outdoor contamination and the remaining part is related to human. This result is consistent with Jaffal *et al*. (1997) and Shekhawat *et al*. (1992). Different commonly used antibiotics were evaluated against all *S. aureus* isolates (Table 1).

Table 1. Antibiotic panel used for *S. aureus*, CoNS, and the percentage resistance of these bacteria to different antibiotics

Antibiotic	Symbol	Concentration (µg)	*S. aureus* (%)	CoNS (%)
Amikacin	AK	3	3.5	9.0
Amoxicillin	AX	25	9.4	9.8
Ampicillin	AM	10	11.3	8.2
Augmentin	AUG	30	-	6.8
Azithromycin	AZM	15	70.0	-
Bacitracin	B	10	-	21.9
Cefaclor	CEC	30	7.5	13.1
Ceftriaxone	CRO	30	-	16.8
Cefotaxime	CTX	30	20.0	11.2
Cefoxitin	FOX	30	33.1	-
Cefuroxime	CXM	30	15.0	16.9
Chloramphenicol	C	30	11.3	7.9
Ciprofloxacin	CIP	5	6.6	6.4
Clarithromicin	CLR	15	50.9	26.9
Cloxacillin	CX	30	-	18.3
Erythromycin	E	15	58.2	19.3
Gentamicin	CN	10	9.4	8.1
Imipenem	IPM	10	4.7	12.5
Linezolid	LZD	30	-	21.8
Novobiocin	NV	30	-	66.9
Ofloxacin	OFX	10	-	8.4
Oxacillin	OX	1	40	-
Penicillin	P	10	13.2	17.9
Rifampin	RA	10	5.2	25.6
Streptomycin	S	300	-	15.1
Tetracycline	TE	30	9.4	12.7
Total			18.0	23.0

S. aureus isolates were resistant to cefoxitin 33.1%, oxacillin 40%, azithromycin 70%, clarithromycin 50.9%, and erythromycin 58.2%.

S. aureus isolates however, showed a remarkable susceptibility (≥ 80%) to amikacin, amoxicillin, ampicillin, cefaclor, cefuroxime, chloramphenicol, ciprofloxacin, gentamycin, imipenem, penicillin, rifampin and tetracycline (Fig. 1A).

S. aureus showed remarkable susceptibility (> 70 %) to all of the tested antibiotics except to novobiocin and rifampin, 30-35% and 65-70%, respectively (Fig. 1A). However, CoNS showed a remarkable resistance (66.9%) to novobiocin (Table 1) but high susceptibility (> 90%) to ciprofloxacin (Fig. 1B).

The results about the profiles of the different antibiotics that are commonly used in Jordan against all *S. aureus* isolates are consistent with the results reported by Shehabi and Baadran (1996) with the exception mainly to novobiocin 66.9%, clarithromicin 26.9%, rifampin 25.6%, bacitracin 21.9% and linezolid 21.8%. The remarkable susceptibility (≥70%) of *S. aureus* isolates to all of the tested antibiotics except novobiocin and rifampin may indicate that these are acceptable alternative antibiotics used for *S. aureus* infection treatments. CoNS in this study showed a remarkable resistance to novobiocin but high susceptibility to ciprofloxacin similarly to a previous study by Saadoun *et al*. (2014), who showed that CoNS were remarkably resistant to novobiocin (~67%), but highly susceptible (~93.5%) to ciprofloxacin.

Maintaining high sanitary standards is important in achieving lower bacterial counts in different wards of the hospitals and designing monitoring strategies should continue to monitor the presence and distribution of CoNS in hospital environments. The extensive use of some antibiotics in hospitals for longer periods may lead to higher resistance percentage of CoNS. Nevertheless, other antibiotics are still acceptable alternatives for CoNS bacterial infection treatments in hospitals and may play an important role to prevent nosocomial infections.

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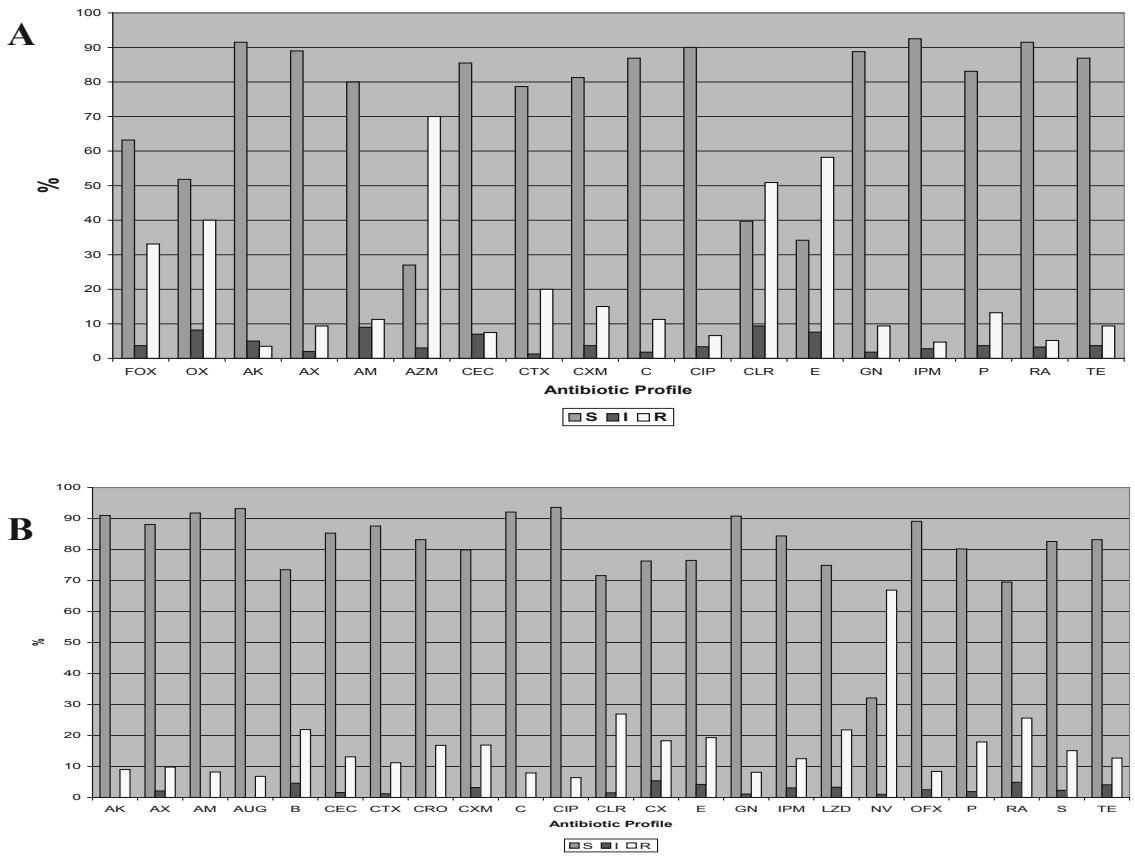

Fig. 1. Antimicrobial susceptibility pattern of (A) *Staphylococcus aureus* and (B) CoNS. **(S)** Susceptible pattern of the antibiotic, **(I)** Intermediate pattern of the antibiotic, **(R)** Resistance pattern of the antibiotic. Amikacin (AK), Amoxicillin (AX), Ampicillin (AM), Augmentin (AUG), Azithromycin (AZM), Cefaclor (CEC), Cefotaxime (CTX), Cefoxitin (FOX), Cefuroxime (CXM), Ceftriaxone (CRO), Chloramphenicol (C), Ciprofloxacin (CIP), Clarithromycin (CLR), Cloxacillin (CX), Linezolid (LZD), Ofloxacin (OFX), Oxacillin (OX), Erythromycin (E), Gentamycin (GN), Imipenem (IPM), Novobiocin (NV), Penicillin (P), Rifampin (RA), Streptomycin (S) and Tetracycline (TE).

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