Antibiotic Susceptibility and Concentration of Airborne Coagulase Negative Staphylococci (CoNS) in the Intensive Care Units (ICU) of Different Hospitals in Northern Jordan

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Abstract

Coagulase negative staphylococci (CoNS) constitute an increasing nosocomial problem especially in hospitals due to intrinsic and acquired resistance to antimicrobials agent. Our objective is to evaluate the CoNS in the adult intensive care units (ICU) and nursery intensive care units (NICU) of Northern Jordan Hospital, to different antibiotics. 128 air samples of 100 liters volume/min were collected by a microbiological air sampler from the above units during the period June to December, 2005. Air samples were impacted on Trypticase soy agar (TSA), and then incubated at 37 ºC for 48 h. Each bacterial colony appeared on agar plates were sub-cultured on TSA or blood agar and incubated at 37 ºC for 24-48 h, and then identified by standard methods. The average bacterial count in the ICU and NICU was 311.7 and 322 cfu/m^3, respectively. CoNS were the most prevalent bacteria of all Gram positives in ICU (26.5%) and NICU (23.5%) with *S. saprophyticus* been the most isolated species (> 65%). CoNS were the most commonly isolated Gram positive cocci in ICU and showed remarkable resistance to Novobiocin (66.9 %), but high susceptibility (> 90%) to Ciprofloxacin.

Intrduction

Indoor air quality of hospitals is remarkably important because the hospital environment is full of microorganisms which may influence the health of people in the hospital and cause nosocomial and other infections through airborne exposure (1). Airborne bacteria are one of these pathogens and evaluation of their count, types and diversity in hospitals rooms especially ICU and NICU is very important to control and prevent nosocomial infections (2 - 4).

Human related organisms from patients, hospital staff and visitors are spread during human activities (5). The contaminated air droplets from patients and staff, outdoor contaminated air (1), number of visitors and the amount of materials brought in from outside are recognized as sources of hospital contamination (3).

About 5–10% of patients admitted to hospitals developed a nosocomial infection and can lead to complications in 25 to 33% of those patients admitted to ICUs, 10 % of the total resulting from airborne microbes. Nosocomial infections may rise from inhalation of droplets in the air or spread by direct hand contact from hospital staff or visitors (6 - 8).

Intensive care units (ICU), and nursery intensive care units (NICU)**,** are considered as sensitive units in any hospital. Most of patients in these units are immuno-compromised patients. The close environment and types of micro-organisms and antibiotics used in these units may provide suitable conditions for the spread of infectious agent's especially multiresistant bacterial pathogens (2, 8). In different studies researchers established that the environment bio-contamination of these hospital units is associated with surgical infections, neonatal sepses and nosocomial infection (3, 9, 10).

The nosocomial infection remains an important problem in intensive care units (8). The number of patients contracting infections in ICUs is particularly high with almost a third of nosocomial infections from respiratory source, but not all of these are airborne since some are transmitted by contact or by intrusive medical equipment (11). Airborne nosocomial infections might also be non-respiratory, for instance with *Staphylococcus* injecting open wounds, burns, or settling on medical equipment (8, 12, 13).

Pediatric and nursery ICUs differ from adult ICUs in a number of ways, apart from the age of their patients. Because pediatric and nursery ICUs are multidisciplinary, and lack physical separations between patients. Therefore, the nosocomial infections represent an important cause of morbidity and mortality in these populations (13). *Javed et al.* (2008), *Chandrashekar et al*. (1997) found that the indoor air of NICU carries large number of common nosocomial bacteria of 4 or more different bacterial species at dangerous levels.

Different microbiological studies done around the world showed that there are different types of micro-organisms which can be transmitted though the air and cause infection in ICU and NICU patients (2, 14).

About One third of microorganisms recovered from hospitals air are Gram Positive Cocci (15); of which CoNS comprise a large group of Gram Positive Cocci at least 18/31 CoNS species have been isolated from human skin (10, 16) of healthy persons and rarely cause infections. However CoNS species are opportunistic pathogens causing infections in immuno-compromised patients, and transmitted from patients and hospital staff through the air (12, 17).

The majority of bacterial species present in the indoor hospital environment is CoNS, and thus may easily contaminate the environment of intensive care units (12). And they have the ability to survive in the ICU surroundings on medical devices and medical equipment for weeks to months (7, 17), This is mainly due to the composition of their wall which contains peptidoglycan resistant to desiccation (18).

If CoNS are present in the air of ICU in large numbers they could colonize in surfaces, equipments, clothes, body of staff and patients, so they are very easily to transmitted by direct contact to patients (17, 19 - 21),

CoNS, especially *S. epidermidis* have emerged as common nosocomial pathogens affecting immuno-compromised patients carrying medical devices, especially intubated ICU patients. Methicillin resistant *S. epidermidis* (MRSE) strains have become also a worldwide problem. A recent Swedish study (18) indicated that MRSE caused 15% of postoperative infections (16, 20).

CoNS, primarily *S. epidermidis*, are specifically prone to cause catheter-related infections. The catheter-related infections are one of the leading causes of nosocomial infections in the ICU setting (18, 21, 22). *S. lugdunensis* has increasingly been recognized as a cause of invasive infections that include endocarditis, osteomyelitis, and sepsis (23).

S. saprophyticus is the second most frequent causative organism of uncomplicated urinary tract infections (UTI) in women (24, 25). The vast majority of infections occur in young, sexually active women. *S. saprophyticus* can also cause UTI in males of all ages (24).

CoNS infections all together compose a serious problem especially among immuno-compromised patients and are often difficult to treat since CoNS strains are commonly multiresistant. In reports from different parts of Europe, the oxacillin resistance in CoNS varies between 70% and 80%, and similar high rates of resistance are also reported from the United States, Canada and Latin America (22, 26, 27). In context, this multiresistance will lead to higher consumption of broad-spectrum drugs such as vancomycin, which increases the antibiotic pressure in the ICU, further promoting the development of antibiotic resistance. It is therefore important to gain more knowledge about colonization, transmission and pathways of dissemination in order to prevent cross-transmission and subsequent nosocomial infections of these bacteria (28).

The pathogenicity of CoNS varied across different species. The most virulent species was *S. lugdunensis* which was associated with clinically significant infections, followed by *S. saprophyticus, S. epidermidis* and *S. haemolyticus* were also clinically significant with low pathogenisity. Most of them were community acquired (20, 23, 28, 29).

For this reason, the objective of this study is to measure the quantities of airborne CoNS bacteria in adult Intensive Care Units (ICU) and Nursery Intensive Care Units (NICU) of Northern Jordan Hospital. The susceptibility of CoNS to commonly used antibiotics was also evaluated.

Materials and methods

The studied hospitals

The study was carried out in north of Jordan over two departments ICU and NICU of four different hospitals serving a population of about 1.3 million.

Collection of air samples

During the period between June and December, 2005, a total of 128 air samples were collected from intensive care units (ICU) and nursery intensive care units (NICU) of four different hospitals in the region of Irbid-Jordan. 16 separated samples were collected from each unit of each hospital.

Each air sample was collected by a microbiological air sampler (M.A.Q.S.II-90 / OXOID, UK) that holds 90 mm Petri dishes within an autoclavable anodized aluminum head of 380 holes. The sampler was set at an air-sampling rate 100 L/min for two minutes per sample. Duplicated air samples were collected from each visit at different sites of each unit with a one meter elevation from the floor (i.e. the same level of the patient's bed).

Sample processing

After impacting the air samples on Trypticas Soy Agar (TSA) media plates, they were transported to the laboratory and immediately incubated at 37 °C for 48 h to determine the total Gram positive cocci bacterial count. Diversity and total counts of bacteria on TSA plates were recorded by using the colony counter (560, Suntex, Labolan).

Bacterial Isolation and identification

Each bacterial colony appeared on agar plates were sub-cultured on blood agar and chocolate agar (Oxoid, UK) to obtain them in pure form. Agar plates were incubated aerobically at 35 °C for 24-48 h. Growth of bacterial colonies was evaluated according to standard methods (30, 31).

Bacterial identification was based on macroscopic and microscopic examination and with specific biochemical tests furthermore. 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 (32). All slides were microscopically evaluated at 100 X.

Based on Gram stain results, each bacterial colony was sub-cultured under aseptic conditions on different culture media for isolation, identification, and testing the susceptibility of the isolates for commonly used antibiotics. The culture media that have been used were: blood agar with 5-7% defibrinized blood, chocolate agar, Mueller-Hinton agar and nutrient agar. All of the above agar or broth media were obtained from Oxoid, UK.

Staphylococcus spp. is a salt-tolerant and catalase positive. The identification of *Coagulase-negative staphylococci (CoNS)* was based on the morphology of colonies, Gram stain, catalase test, growth on mannitol salt agar and the negative reaction to coagulase production which was performed to the standard methods (33).

Plates and inoculums preparations for antimicrobial susceptibility test

The turbidity of the test organisms was adjusted to be equal to or greater than 0.5 McFarland turbidity standards (1.5×10^8 CFU/ml) and inoculated by using a sterile cotton swab on the surface of two freshly prepared Mueller-Hinton agar plates. The plates were incubated at 37 °C for 24 hours. Mueller-Hinton agar plates and 0.5 McFarland standards were prepared according to the recommendation of the Kirby-Bauer disc diffusion procedure (22) and NCCLS (34).

Application of disks and incubation

Twenty one different antibiotics discs (Oxoid, UK) were used for susceptibility testing of CoNS. Antibiotic disks were applied to the agar surface by a sterile forceps and then gently pressed to ensure complete contact of the disks on the agar surface. Plates were inverted and incubated at 35°C for 16-18 hours. The results of susceptibility testing (inhibition zone diameter) for each isolate were measured in (mm) and interpreted according to NCCLS standards on a special data sheet.

Statistical analysis:

Data was processed using of Statistical Package for Social Sciences (SPSS) statistical software. Comparisons between groups (hospitals and units) were tested using one way ANOVA test and thereafter between each two independent samples was done using T-test. Two tailed P values < 0.05 was considered significant.

Results and discussion

Number and diversity of isolated bacteria

Airborne Bacteria are the major types of microorganisms present in all hospital environments (10, 15). Table 1 shows the bacterial count in ICU and NICU ranged between 30 and 1160, and 40 and 1130 cfu/m³, respectively. Similar results were reported, the microorganism's average in ICU was found to be 687 cfu/m³ (5) While diversity of bacteria were ranged between 2 and 23, 2 and 25, respectively (Table 1). Total air bacterial count and diversity in ICU and NICU units were not significantly different (P-value 0.258). The wide range of bacterial counts in ICU and NICU (Table 1) could be explained by many factors including the high number of visitors, cases of patients, opened doors and the amount of material brought from outside through flowers and fruits (1, 3, 8). These were recognized as sources of hospital contamination (15, 31). Diversity of bacteria is usually related to the count. The bacterial diversity was found to be similar in the different hospital units under study. It was accepted that the high microbial biodiversity is associated with high temperature and relative air humidity that favors microbial growth (2, 19). Similar to our results, Beggs (2003) reported that approximately two thirds of microorganisms isolated from the hospitals air were gram positive cocci.

Isolation and identification of bacteria

The results of cultured sample identification showed three major groups of bacteria: gram positive cocci (91 %), gram positive bacilli (2 %), gram negative bacilli 5.5 % and unidentified bacteria 1.5% (Table 1). *Staphylococcus* was the most isolated Gram positive cocci bacteria (78%); of which CoNS

comprised (50%) of all isolated *Staphylococcus* and distributed as 26.5% in the ICU and 23.5% in the NICU as shown in Figure 1. The markedly high occurrence of CoNS bacteria in ICU may be related to the high number of visitors and to the outdoor contamination. Other source of contamination could be related such as human activities, bacterial structure (7, 8, 15). *Obbard* (2008) showed a large number of bacteria carrying particles in air of NICU and pediatric ward. Also he showed that there is a direct relation between floor area per person and bacterial contamination of air. Previous reports indicated that 44% of all isolated bacteria from the NICU were *Staphylococcus* (12). Newman (2002) showed that 43% of isolated bacteria from air of NICU were CoNS.

Table 1. Number and Diversity of isolated bacteria (CFU/m³) in intensive care units (ICU) and nursery intensive care units (NICU) of different Hospitals in Irbid, Jordan**.**

Bacteria (Number, Diversity and Type)	Intensive Care Units (ICU)	Nursery Intensive Care Units (NICU)
Number of bacteria		
Range	30-1160	40-1130
Average	311.7	322
Diversity of bacteria		
Range	2-23	2-25
Average	10.6	12
Groups of bacteria	Percentage (%)	
Gram positive cocci	91	
Gram positive bacilli	2	
Gram negative bacilli	5.5	
Unidentified bacteria	1.5	

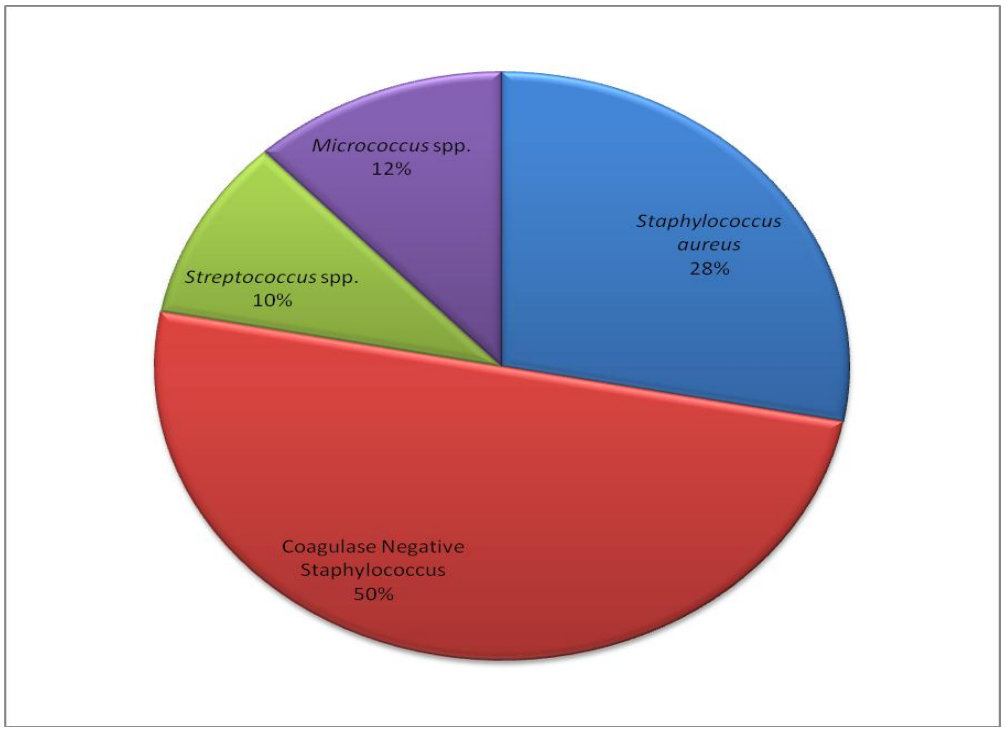

Figure 1: Percentage distribution of Gram Positive Cocci bacteria

It was found that *S. saprophyticus* (Novobiocin resistante) which comprises 66.9% from all coagulase negative *Staphylococcus* (CoNS), while the other *Staphylococcus* spp. comprised the other 33.1%. These results indicated that the major part of CoNS in the hospital air is related to outdoor contamination and the remaining part is related to human. The present findings are consistent with other studies (2, 5, 35).

Antimicrobial susceptibility profiles

The different antibiotics commonly used in Jordan were evaluated against all coagulase negative *Staphylococcus* isolates by disc diffusion method. CoNS isolates showed high resistance to Novobiocin 66.9, Rifampin 25.6%, Linezolid 21.8%, Erythromycin 19.3%, Cloxacillin 18.3% and Penicillin 17.9% (Figure 2). These results are consistent with other results reported in literature (24, 29, 30, 36)**.** However, CoNS in this study showed a remarkable susceptibility of (≥ 90%) to Ciprofloxacin, Chloramphenicol, Gentamycin, Ampicillin, Ofloxacin, Amikacin and Amoxicillin as show in Fig. 2. This is consistent with finding in different studies (30, 35, 36, 37). Thus, it can be concluded these are acceptable alternative antibiotics that can be used for CoNS infection treatment.

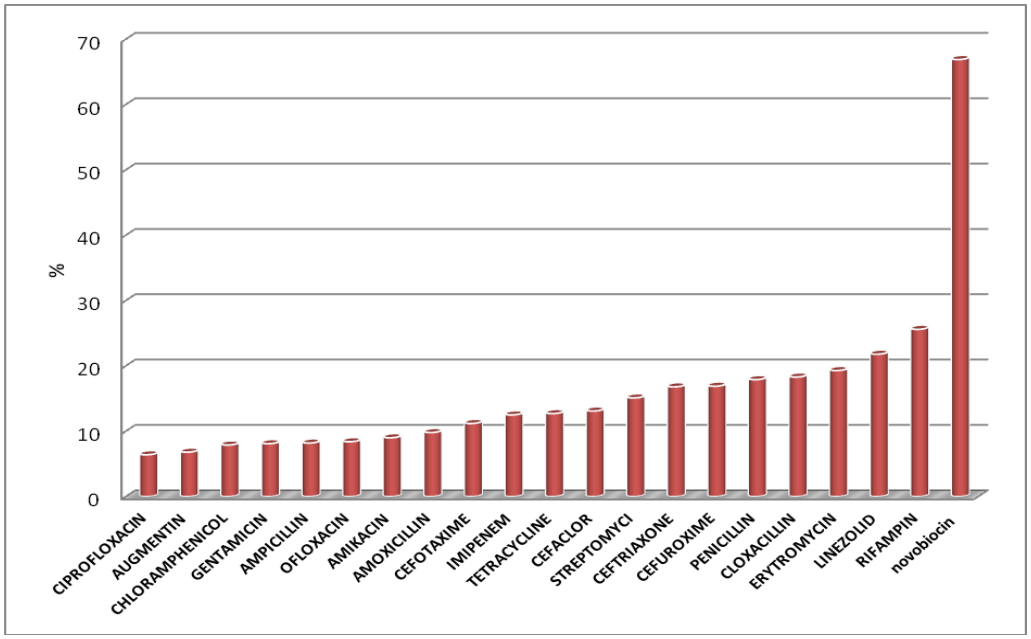

Figure 2: Antimicrobial resistance pattern of Coagulase Negative *Staphylococcus* (CoNS) bacteria

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دراسة تركيز بكتيريا المكورات العنقودية السالبة لفحص التخثر (CoNS) المحمولة في هواء غرف العناية الفائقة (ICU) ومدى حساسيتها للمضادات الحيوية في عدة مستشفيات، شمال الاردن

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الملخص

إزداد الإهتمام مؤخرا بإصابات المستشفيات وخاصة الناتجة من بكتيريا المكورات العنقودية السالبة لفحص التخثر (CoNS) وذلك بسبب مقاومتها الذاتية والمكتسبة للأدوية المضادة للجراثيم.

تهدف هذه الدراسة لتقييم وجود وانتشار بكتيريا (CoNS) في غرف العناية الفائقة الخاصة بالبالغين (ICU) والمواليد الجدد (NICU) في مستشفيات شمال الاردن ومدى حساسية هذه البكتيريا للعديد من المضادات الحيوية.

لقد تم جمع 128 عينة هواء بواقع 100 لتر في الدقيقة بواسطة جهاز خاص من غرف العناية الحثيثة بنوعيها في الفترة الواقعة ما بين شهر 6 - 12 / 2005، وقد تم زراعة هذه العينات على وسط Trypticase soy

agar (TSA) ثم تم حضانة الاطباق على درجة حرارة 37 درجة مئوية لمدة 24 ساعة، وبعد ذلك تم عزل كل مستعمرة جرثومية تظهر على الأطباق بشكل نقي على وسط TSA او وسط آجار الدم وحضنها على درجة حرارة 37 درجة مئوية لمدة 24 - 48 ساعة ليتم تشخيصها بالطرق المثالية المتبعة في التشخيص.

وقد بلغ عدد البكتيريا في ICU وNICU ما معدله 311.7 و 322 في كل متر مربع من الهواء على التوالي، وكانت بكتيريا CoNS هي الأكثر شيوعا وشكلت النسبة الاكبر بين البكتيريا المعزولة، حيث ظهرت بنسبة 26.5 % في ICU وفي NICU شكلت ما نسبته 23.5 %، وقد شكل النوع *S. saprophyticus* النسبة الاكبر من بين انواع هذه البكتيريا بنسبة تجاوزت 65 %، حيث اظهرت هذه البكتيريا مقاومة مرتفعة للمضاد الحيوي Novobiocin بنسبة 66.9 %، بينما كانت حساسة للمضاد الحيوي Ciprofloxacin بنسبة تجاوزت 90%.

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