RESEARCH ARTICLE

STETHOSCOPES AS VECTORS OF MULTI-RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI IN A TERTIARY HOSPITAL

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ABSTRACT

Background: Stethoscopes are an essential tool for physicians, but can be a source of hospital infections.

Aims & Objective: The objective was to isolate staphylococci strains from the diaphragms and earpieces of the stethoscopes as well as to characterize their antibiotic resistance.

Material and Methods: Forty five stethoscopes from different clinical doctors were sampled in a tertiary hospital. The swab method was used and the swabs were immediately inoculated on blood agar and manitol salt agar. The bacterial identification and the antibiotic resistance were performed according to the CLSI with automated commercial system.

Results: Pathogenic or potential pathogenic Coagulase Negative Staphylococci (CoNS) were isolated on physician's stethoscopes. CoNS were isolated in 87.6% of the stethoscopes. S.epidermidis was the predominant isolate species of CoNS (39.4%) followed by S.hominis (19.7%), S.auricularis (9.8%), S. haemolyticus (8.4%), S.warneri (7%), S. cohnii (5.6%), S. lugdunensis (4.2%), S. capitis (4.2%), S.hyicus (1.4%). The isolated CoNS have high resistance rate in penicillin (74.6%), antibiotic macrolidess (60.5%), clindamycin (39.4%), oxacillin (30.9%) and gentamycin (22.4%). The co-resistance of S.epidermidis for methicillin-gentamicin was 59.2%. Multi-resistant strains of CoNS were 16.9% and belong to the species of S.epidermidis, S.hominis and S.haemolyticus.

Conclusion: The study demonstrates the contamination of the stethoscopes with different species of CoNS which showed high antibiotic resistance and possess the potential risk of causing hospital infections. As severe hospital infections, especially in population at risk caused by different species of CoNS, are increasing; their characterization at the species level as well as their resistance pattern in patients and medical devices is strongly suggested.

KEY-WORDS: Antibiotic Resistance; Coagulase-Negative Staphylococci (CoNS); Contamination; Hospital Infections; Stethoscopes

Introduction

Infection transmission in the hospital environment (nosocomial infection) remains a significant hazard for hospitalized patients. Health-care workers are potential sources of these infections. Many pathogens can be transmitted on the hands [1], on contaminated medical devices such as electronic thermometers, blood pressure cuffs, stethoscopes, latex gloves, masks, neckties, pens, badges and lanyards, and white coats.[1-6] Few published studies refer to the contamination of stethoscopes diaphragms, that are in contact with patient bodies.[6-12] Apart from diaphragms, earpieces merit the same attention because they are frequently colonized with microorganisms either by the hands of the users or by the patients ear canals.[13,14] Stethoscopes are commonly used to assess the health of patients and have been reported to be potential vectors for nosocomial infections in various parts of the world.[7,15,16] Following contact with infected skin, pathogens can attach and grow on the diaphragms of stethoscopes and subsequently be transmitted to other patients if the stethoscope is not disinfected.[17,18] The predominant bacteria isolated from stethoscopes, in most studies, are coagulase negative staphylococci (CoNS).[7,8] Some studies refer S. aureus and Gram-negative bacteria as severe bacterial contamination of stethoscopes and neglect the presence of the different species of CoNS. As every day new species of CoNS are responsible for severe nosocomial infections, stethoscopes may be responsible for the dissemination of these bacteria.

The objectives of this study were (i) to investigate the CoNS contaminated physicians stethoscopes

(diaphragms and earpieces), (ii) to discriminate in the species level (iii) to determine the antibiotic sensitivity of bacterial isolates from stethoscopes (iv) to outline the public health implications of stethoscope contamination.

Materials and Methods

This study was performed from November 2010 to February 2011 in a tertiary hospital (Athens Medical Center) with 250 beds. Stethoscopes of 45 physicians from different medical departments of the hospital as well as different specialties were included to the study (Table 1). Samples from diaphragms and earpieces from each physician's stethoscope were obtained. The diaphragms and the entire surface of the earpieces (including orifices) were swabbed with cotton swab moisture with sterile saline solution and immediately inoculated onto different blood and manitol salt agar plates (Oxoid). The inoculated plates were incubated at 37^0C for 24-48 hours. Then, the colonies were subcultured for the identification of the isolated bacteria. By evaluating the colony characteristics and also by using Gram stain, the isolated bacteria were identified. Afterwards, for the identification of CoNS, 25 biochemical reactions (Table 2) were included in the data of commercial automated Siemens MacroScan Walk Away 96 system (West Sacramento CA 95691), a system which is used routinely in the laboratory. Antibiograms were also performed by using the same automate commercial system MicroScan (Siemens). According to the CLSI break points, bacteria were categorized as sensitive, resistant or intermediate.[20] The manufacturer's panels by CLSI included 20 antibiotics belonging into 15 different classes proposed.

Results

Forty five stethoscopes used by different physicians from different clinics were sampled (Table 1). 55.6% of the physicians were men. The average age of the physicians was 56 years old. Our results showed that 90% of the physician's stethoscopes were contaminated with pathogenic and potential pathogenic bacteria. CoNS were the predominant isolates (87.6%). The rest were Acinetobacter lowffi, Streptococcus faecalis and Streptococcus sanguinis. As far as CoNS species isolates are concerned, S.epidermidis was the predominant one (39.4%) followed by S. hominis (19.7), S. auricularis (9.8%), S.haemolyticus (8.4%), S.warneri (7%), S. cohnii (5.6%), S.lugdunensis (4.2%), S.capitis (4.2%), S.hyicus (1.4%) (Table 3). The frequency of CoNS species isolated by ear and diaphragm is recorded in Table 3. The percentage of resistance of CoNS isolated from both the diaphragms and earpieces, to antibiotics tested according to CLSI, is shown in Tables 4 and 5. From 71 isolated CoNS strains, 74.6 % were resistant to penicillins (producing β-lactamase) and 30.9 % were resistant to oxacillin (cefoxitine screen positive). A high resistance rate (60.5%) was also reported for macrolidess (erythromycin-clarythromycin), clindamycin (39.4%), gentamicin (22.4%). Fewer of the isolates were resistant to levofloxacin (14%), trimethoprim/ sulfomethoxazole (14%), tetracycline (8.4%) and rifampin (2.9%). No resistance was found to teicoplanin, linezolid, daptomycin, vancomycin, chloramphenicol, quinupristin-dalfopristin, fucidic acid. The antibiotic resistance rate of different species of CoNS isolated from stethoscopes diaphragms and earpieces is shown in table 3. S.epidermidis, S. hominis and S.haemolyticus were the most resistant species of CoNS. The co-resistance of S.epidermidis for methicillin-gentamicin was 59.2%.

Although no clear-cut definition exists for the classification of multiresistance, isolates were considered to be multiresistant when their growth was not inhibited by at least four classes of the antibiotics tested. From 71 CoNS, multi-resistant species of CoNS were found in 16.9% of the isolates (6 S. epidermidis, 4 S. Hominis and 2 S. haemolyticus). Three out of five S. warneri were resistant to penicillin and erythromycin, one was penicillin-erythromycin and methicillin resistant and one resistant only to erythromycin S. capitis and S. hyicus were the most sensitive species. Four out of seven strains of S. auricularis and two S. cohnii produce β-lactamase (Table 5). Six strains of 71 CoNS (8.4%) were resistant to tetracycline (4 S. hominis and 2 S. haemolyticus) and 10 strains (14%) were resistant to Trimethoprim/ Sulfamethoxazole (4 S. epidermidis, 4 S. hominis and 2 S. haemolyticus).

Table-1: Specialty and Sex of Physicians who's Stethoscopes Belonged

Specialty	Men	Women	% of Total
Anaesthesiologist	0	2	4.4
Cardiologist	1	0	2.2
Gastroenterology	1	0	2.2
Intensive Care Unit	4	0	8.9
Neurology	1	2	6.7
Pathology	12	6	40
Paediatrician	2	6	17.8
Pneumonology	0	3	6.7
Surgery	4	1	11
Total	**25**	**20**	

Table-2: Biochemical Reactions for Staphylococcus spp. Identification by Microscan WalkAway 96

• Growth in the presence of low concentration of crystal violet (CV)
• Growth in the presence of 0.05 µg/ml bacitracin (BAC)
• Reduction of nitrate to nitrite (NIT)
• Acetylmethylcarbinol production from glucose (VP)
• Presence of pyrolidonyl-arylamidase (RYR)
• Production of specific glycosidase enzyme (PGT)
• Production of acid under aerobic growth condition from lactose (LAC), trehalose (TRE), mannose (MAS)
• Tolerance to 6.5 % sodium chloride (NACL)
• Susceptibility to low concentration of bacitracin (BAC)
• Susceptibility to optoclin (OPT)
• Presence of urease (UREA)
• Growth in the presence of low concentration (1.6 µg/ml) of monobiocin (NOV)
• Production of the enzyme indoxyl phosphatise (IDX)
• No growth in the presence of 40% bile esculin (BE)
• Dehydrolization of arginine (ARG)
• Fermentation of carbohydrates manitol (MAN), sorbitol (SOR), arabinose (ARA), raffinose (RAF), inulin (INU), ribose (RIB)
• Presence of alkaline phosphatise (PHO)
• Utilization of pyruvate (PRV)

Table-3: Percentage of Coagulase-Negative Staphylococcus Species Isolated from Ear and Diaphragm of Stethoscopes.

Staph Species	Ear	Diaphragm
S. auricularis	8.9	6.7
S. capitis	2.2	ND
S. cohnii	6.7	4.4
S. epidermidis	17.8	44.4
S. haemolyticus	11.1	6.7
S. hominis	20.0	17.8
S. hyicus	2.2	ND
S. micrococcus	2.2	ND
S. morexella	2.2	ND
S. sauguinis	2.2	ND
S. warneri	6.7	4.4
S. lugohinensis	ND	2.2
S. lugdunensis	ND	4.4
S. alowfich	ND	2.2
NG	17.8	6.7
Total	**100.0**	**100.0**

ND: not detected

Table-4: Antimicrobial Resistance of Coagulase Negative Staphylococci Isolated from Stethoscopes (Diaphragms and Earpieces).

Antibiotics	No. of Resistant CoNS (Total No. 71)	%
Ampicillin	53	74.6
Amoxicillin/Clavulanic acid	28	39.4
Cephalothin	22	30.9
Erythromycin	43	60.5
Clindamycin	28	39.4
Levofloxacin	10	14
Gentamicin	16	22.5
Oxacillin	22	30.9
Penicillin	53	74.6
Rifampin	2	2.8
Tetracycline	6	8.4
Trimethoprim/ Sulfamethoxazole	10	14

No resistance was found to teichoplanin, linezolid, daptomycin, vancomycin, chloramphenicol, quinupristin-dalfopristin, fucidic acid.

Table-5: Antimicrobial Resistance of Different CoNS in Species Level Isolated from Stethoscopes (Diaphragms and Earpieces)

Antibiotics	S. epidermidis (28)	S. hominis (14)	S. warneri (5)	S. lugdunensis (3)	S. auricularis (7)	S. cohnii (4)	S. capitis (3)	S. haemolyticus (6)	S. hyicus (1)
Ampicillin	26	12	3	0	4	2	1	4	1
Amoxicilin/ clavulanic acid	14	8	1	0	3	2	0	2	0
Cephalothin	14	6	1	0	0	1	0	0	0
Levofloxacin	7	2	0	0	0	0	0	0	0
Clindamycin	22	4	0	0	0	0	0	2	0
Erythromycin	24	10	5	0	2	2	0	0	0
Gentamicin	10	6	0	0	0	0	0	0	0
Oxacillin	14	6	1	0	0	1	0	0	0
Penicillin	26	12	3	0	4	2	1	4	1
Tetracycline	0	4	0	0	0	0	0	2	0
Trimethoprim/ Sulfamethoxazole	4	4	0	0	0	0	0	2	0
Rifampin	2	0	0	0	0	0	0	0	0

Discussion

The results of this study revealed that most diaphragms and earpieces stethoscopes used by physicians are contaminated with CoNS. This is in agreement with the findings of Marinella et al.[9], who showed that physicians' stethoscopes have higher bacterial load compared with the stethoscopes of other health care workers. In our study, men stethoscopes were more contaminated than women physicians stethoscopes. Also, the age as well as the specialty of the physician was

statistically important for the stethoscope microbiological burden. There is no previous reference, as far as we know, to correlate the contamination with these two factors.

CoNS were isolated on the 87.6% of stethoscopes, in line with what has been reported by many similar studies.[6-8,10] Similarly to Shobha et al.[19], no S.aureus was isolated from the diaphragms and earpieces stethoscopes examined. Five out of nine CoNS species (S. epidermidis, S. hominis, S. haemolyticus, S. lugdunensis and S.warneri) isolated from physicians stethoscopes in our study have also been isolated from clinical specimens of documented infections in our hospital. This observation reinforces the notion that at least these species are part of the hospital population.

Patients become colonized with pathogenic or potential pathogenic bacteria after contact of already contaminated hands, accessories, gloves or equipment.[8] Most physicians understand the usefulness of routine cleaning of the stethoscopes, but they usually clean the diaphragms and not the earpieces. Some physician would argue that it has been documented that eradication of bacteria from earpieces after their cleaning is hard because of their design. For this reason, in our study we swabbed not only the surface of the earpieces but also the inside orifice. The contamination of stethoscopes earpieces poses some risks to the stethoscopes users. The reported case of a nurse who developed external otitis caused by S. aureus after extensive use of a stethoscope carrying the same microorganisms confirms the fact that colonization of stethoscopes earpiece could cause infection not only to the patient but also to the users.[13] Given the polymicrobial nature of otitis externa it is possible that all species of bacteria isolated from stethoscopes earpieces cause infection of the ear canal of the user, especially when concomitant risk factors coexist (diabetes, dermatitis, blood disorders etc.). The study of Roland and Storman[20] who isolated different species of CoNS and gram negative bacteria from acute external otitis confirm this hypothesis.

According to Cunha et al.[21], CoNS species identification is very important because certain species are associated with nosocomial infections. In low-weight newborns, S. epidermidis have been considered the main cause of hospital infections.[22,23] Nowadays other species such as S. cohnii, S. haemolyticus, S. lugdunensis, S. warneri and many others are associated with hospital infections, especially in ICU wards.[24-31] No data are available for the contamination of the species of CoNS on stethoscopes identified in species level and determinate of the antimicrobial resistance pattern of these species. These previous "neglected" cocci, because of their genome flexibility, can generate novel phenotypic and genotypic variants enhancing their virulent and antibiotic resistance.

CoNS isolated from physicians' stethoscopes are highly resistant to penicillin (74.6%) and microlides (60.5%), gentamicin (22.4%) and levofloxacin (14%). High methicillin resistance carriage of CoNS in stethoscopes was shown in the study of Shobha et al.[19] CoNS species methicillin resistance should confront with caution for possible appearance of multiresistance.

In our study 12 CoNS (6 S. epidermidis, 4 S. hominis and 2 S. haemolyticus) were multiresistant and 10 were methicillin resistant. The propensity of the different CoNS species to acquired multi-resistance and the fact that they are extremely virulent for population at risk, emphasize the importance of their antibiotic resistance determination. There are some discrepancies of the multi-resistance pattern in different studies because of luck of definition of multiresistance. The simultaneous presence of resistance to methicillin-gentamicin of S. epidermidis in our study is 59.2% in agreement with the study of Koksal et al.[32] which showed the simultaneous presence of mec A gene and at least one or more aminoglycocide modifying enzyme in staphylococci isolated from different clinical specimens using multiplex polymerase chain reaction (PCR). All multiresistant species of CoNS present on stethoscopes could be spread to nosocomial populations and cause many kinds of problems to hospital infections programmes. S. epidermidis multi-resistant strains are well documented as the predominant species of CoNS isolated from hospital infections in population at risk.[20] Multi-resistant strains of S. hominis (subsp. hominis) have been isolated from nosocomial blood stream infections

predominantly from ICU.[13] S. lugdunensis is an unusual virulent CoNS and have been implicated in a variety of severe infections (abscess, wound infections, endocarditis, otitis, implanted medical devices) that resemble S. aureus and many times had been misidentified as S. aureus (if we had used a commercial clumping factor). The plethora of case reports published on S. lugdunensis infections shows that this species of CoNS should be considered as serious as S. aureus infections.[14] S. haemolyticus is responsible of blood stream infections in newborns and adults often with high resistance antibiotics rate.[6] S. warneri, although not a predominant pathogen, can cause hospital infections in populations at risk like neonates in NICUs linked with the colonization of nurses hands.[7]

Also it has been found to cause serious orthopedic infections usually in association with the presence of implants materials and catheter related bacteremia.[15] S. cohnii is rather easy to treat unless mutated like the multiresistant strain of S. cohnii, resistant also to linezolid. It was isolated in blood culture of patients in ICU in Greece.[28]

S. capitis is part of the normal flora of skin and ears and it has been associated with prosthetic valve endocarditis.[18] S. auricularis has rarely been implicated in blood stream infection.[21] S. hyicus has not been reported to cause human infections but only to animals. However a wound infection has been reported by Osterlund and Nordlund after a donkey bite.[21]

Conclusion

In conclusion identification of the CoNS species level that contaminated stethoscopes, as well as their antimicrobial resistance pattern is crucial in order to convince the physicians and other workers for the necessity of their compliance to health hygiene (cleaning hands, stethoscopes and other objects). This is the only way to control the dissemination of hospital infection.

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