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Research article

Distribution of pathogenic microorganisms isolated from dental hospital workers in Korea

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ABSTRACT

With the significant rise in hospital infection management in dental hospitals as well as in hospitals, and in order to identify the distribution of pathogenic bacteria on hands and nasal cavity of workers in a dental hospital, bacteria from the hands and nasal cavities of six dentists and 44 dental hygienists from four dental hospitals were investigated. The results showed *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus xylosus*, *Staphylococcus. Lugdunensis*, and *Neisseria spp.*, were isolated from the nasal cavity and *Staphylococcus aureus*, *Staphylococcus capitis*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus xylosus*, *Staphylococcus leutus*, *Micrococcus spp.*, *Staphylococcus cohnii*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pseudomonas pneumotropica* from the hands. An antimicrobial disk diffusion test was conducted on *Staphylococcus aureus* isolated from the hands and nasal cavity to detect MRSA by means of oxacillin. Two strains were detected. When the genes of penicillin binding protein 2 (*mec*A) were detected from the 2 strains, MRSA was found from both strains. The results of this investigation on the distribution of various pathogenic bacteria and MRSA on hands and nasal cavity of workers of a dental hospital, will contribute to the basic data for the future infection management in a dental hospital.

Keywords: dental hospital worker, dentist, dental hygienist, methicillin-resistant *Staphylococcus aureus*, antimicrobial susceptibility, *mec*A

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INTRODUCTION

There are many resident and pathogenic bacteria on gowns, ties, hands, and other devices that hospital workers use. Bacteria can also be found on patients sleeping gear and in a dental hospital, which is similar to a hospital in its environment, these can be polluted by various secretions from dental treatments.[1,2] Bacteria spread to patients from hospital treatment staff and vice versa. The main infectious bacteria found in hospitals are *Staphylococcus aureus* and Coagulase-Negative Staphylococci (CNS), which have been frequently detected on hospital worker's clothes, hands and nasal cavities. When these bacteria are carried to a patient who has a low immunity against infectious bacteria they can cause disease.[3] Among the bacteria isolated in hospitals, *S. aureus* resides on the skin and soft tissues and can cause an infectious disease to a patient with a weak immune system.[1,3] Methicillin-resistant *S. aureus* (MRSA) was generated from the senseless abuse of antibiotics, and according to a recent report, MRSA infection rate among children is increasing, causing infectious diseases in children as their immune system is low.[4,5] Recently, as more attention is being paid to infectious bacteria in dental hospitals, dental unit water systems and unit chairs are suspected to play a role in bacterial infection, and the distribution and infection degree of bacteria on dental unit water system used by patients in a dental hospital have been investigated.[6] Dental health care workers investigated the possibility of infection from the Hepatitis B virus and *Mycobacterium tuberculosis*. *Mycobacterium tuberculosis* infections among the dental hospital patients passed to the dental health care workers through the infectees' saliva and blood. The isolation rate of *S. aureus* on hands and nasal cavities of dental hygienic students, who are to-be dental hospital workers, were 23% and that of MRSA from *S. aureus* was 20%.[7,8] Today, according to national research reports, there is information on the infectious diseases of dental hospital patients and pathogenic bacteria from dental hygienic students who are to-be dental hospital workers, but there little data on the infection degree of resident bacteria on the hands of dental health care workers who directly contact dental hospital patients. Our aim is to prepare basic data on dental hospital infection management through the identification of the distribution of pathogenic bacteria on hands and in the nasal cavities of dental health care workers and provide an investigation into the infection degree of MRSA and Vancomycin-resistant *S. aureus* (VRSA), which are the most problematic.

MATERIALS AND METHODS

Samples

In Daegu, Korea, 2001, pathogenic microorganisms on the hands and in the nasal cavities of six dentists and 44 dental hygienists, from four dental hospitals, were isolated.

Isolation and identification of pathogenic bacteria

For the isolation of pathogenic bacteria, samples from hands and nasal cavities of 50 dental health care workers were collected with a sterilized cotton bud, then cultivated in a blood agar plate (Becton, Dickinson and Company, USA) and Mannitol salt agar (Becton, Dickinson and Company, USA).[9,10] The cultivation condition was aerobic, at 35°C for 24 h. After gram stain with the use of purely isolated clusters, the O.D value of the clusters was set to 600 nm in 0.45% saline, and bacterial liquid was injected into the identification card, designed for bacteria identification for cultivation, at 35°C for 24 h.[11] The specific name of bacteria was identified by means of the VITEK system, which is an automatic device for bacteria identification and 16s rRNA.[12,13]

Antibiotic susceptibility test

Antimicrobial disk diffusion test was conducted on *S. aurues* by means of VITEK.[14] After the identified *S. aurues* was inoculated to Mueller Hinton agar (Becton, Dickinson and Company, USA), MRSA was defined with Oxacillin.[15] For *S. aureus*, ATCC 29213 as the negative control, MRSA ATCC 33593 as the positive control and MRSA, which was identified by antimicrobial disk diffusion test. The antimicrobial susceptibility test was conducted with the use of VITEK system, and the types of antimicrobial were Benzylpenicillin, Cefoxitin screen, Ciprofloxacin, Clindamycin, Erythromycin, Fusidic Acid, Gentamicin, Habekacin, Inducible Clindamycin Resistance, Linezolid, Mupirocin, Nitrofurantoin, Oxacillin, Quinupristin/Dalfopristin, Rifampicin, Teicoplanin, Telithromycin, Tetracycline, Tigecycline, Trimethoprim/Sulfamethoxazole and Vancomycin. The result of the antimicrobial susceptibility test was defined as 2011, Clinical and Laboratory Standards Institute Guidelines.[16]

mec A gene PCR of MRSA strain

Among the clusters cultivated at 35°C for 24 h after being inoculated to Mannitol salt agar (Becton, Dickinson and Company, USA), a single cluster was drifted with a TE buffer of 50 μl. The suspension was boiled for 10 min at 100°C, centrifuged at 12,000 rpm for 10 min and underwent PCR with the use of supernatant liquid. Primer was *mec* A Forward 5′- TGG CTA TCG TGT CAC AAT CG-3′ and *mec* A Reverse 5′-CTG GAA CTT GTT GAG CAG AG-3′.[2,3] Reaction buffer was 10 * buffer 5 μl, 2.5 mM dNTP 4 μl, Taq polymerase 1.25 U and DNA template 10 μl. Reaction condition was 94°C, 3 min denaturation, then 94°C for 30 s, 45°C for 30 s, and 72°C for 1 min, repeated 30 times. The final extended reaction appeared at 72°C for 10 min, and the product of the reaction was found after electrophoresis for 30 min at 100 V in 1% agarose gel.[17,18]

Analysis of nucleotide sequence of *mec* A

For an exact analysis of the nucleotide sequence of MRSA products of the PCR were identified by requesting nucleotide analysis at SolGent, Korea, a nucleotide sequence analyzing company. Homogeny of the nucleotide sequence was investigated with the use of penicillin binding protein 2 (*mec*A, Accession No. JN108029) of *S. aureus* and GeneDoc program.

RESULTS

Isolation and identification of pathogenic bacteria from the nasal cavity

From the nasal cavity, 48 bacterial species of gram positive cocci and 2 bacterial species of gram negative cocci were isolated. As a result of identification of the isolated bacteria, *S. aureus*,[11] *S. capitis*,[12] *S. epidermidis*,[18] *S. hominis*,[6] *S. warneri*,[9] *S. xylosus*,[2] and *S. lugdunensis*[12] were isolated as gram positive cocci, and *Neisseria spp.*[17] were isolated as gram negative cocci. The most frequently isolated species were *S. xylosus*,[2] *S. aureus*[11] and *S. epidermidis*,[18] in the order of isolation times.

Isolation and identification of pathogenic bacteria from hands

From hands, 76 bacterial species of gram positive cocci and 8 bacterial species of gram negative cocci were isolated. *S. aureus*,[3] *S. capitis*,[6] *S. epidermidis*,[19] *S. hominis*,[20] *S. warneri*,[13] *S. xylosus*,[21] *S. leutus*,[9] *Micrococcus spp.*,[6] and *S. cohnii*[12] were isolated as gram positive cocci and *Serratia marcescens*,[17] *Pseudomonas aeruginosa*,[9] *Klebsiella pneumonia*,[17] and *Pseudomonas pneumotropica*[12] were isolated as gram negative bacilli. Among them, the most frequent isolated bacterial species were *S. xylosus*,[21] *S. epidermidis*,[19] *S. aureus*,[3] and *S. hominis*,[20] in the order of isolation times.

Antimicrobial susceptibility test of MRSA strains

Antimicrobial disk diffusion test was conducted on *S. aureus*[11] isolated from nasal cavities and *S. aureus*[3] isolated from hands. The isolated *S. aureus* was inoculated to Mueller Hinton agar, and then MRSA was defined with the use of Oxacillin. As a result, *S. aurues*, which is resistant against Oxacillin, was found to be in two strains from both nasal cavities and hands. Each of two strains were isolated from the same person's nasal cavity and hands. By means of *S. aureus*, ATCC 29213 as a negative control, MRSA ATCC 33593 as the positive control and the two strains that were defined to be MRSA by the antimicrobial disk diffusion test. An antimicrobial susceptibility test was conducted to investigate the degree of resistance and susceptibility of antibiotics other than Oxacillin. Accordingly, the two MRSA strains were found to be resistant against Benzylpenicillin, Cefoxitin screen, Oxacillin, Tetracycline, Fusdic acid, and Mupriocin and sensitive to the other antibiotics (Table. 1).

Detection of PCR product of *mec* A of MRSA strains

As a result of an antimicrobial disk diffusion test using Oxacillin, PCR was conducted on two strains which were defined as MRSA, to identify whether the gene of *mec* A was detected or not. Consequently, *mec* A was detected from both MRSA strains isolated, from the positive control MRSA ATCC 33593 and dental health care workers (Figure 1).

Analysis of nucleotide sequence of gene of *mec* A of MRSA strains

For an exact analysis of nucleotide sequence of *mec* A gene, the products of PCR were identified by requesting nucleotide sequence analysis at SolGent, Korea, and homology of the identified base grade

Table 1. Antimicrobial susceptibilities test of MRSA 3rd and 9th strains isolated from dental hospital workers using the VITEK system.

	VITEK system			
	S. aureus ATCC		No. 3	No. 9
Antibiotic (MIC; μg/ml))	MIC (MIC; μg/ml))	Result	Result	Result
Cefoxitin Screen(Neg/Pos)	POS	NEG	POS	POS
Benzylpenicillin (<= 0.03)	>= 0.5	S	R	R
Oxacillin (<= 0.25)	>= 4	S	R	R
Gentamicin (<= 0.5)	>= 16	S	S	R
Habekacin (<= 0.1)	<= 1	S	S	S
Ciprofloxacin (<= 0.5)	<= 0.5	S	S	S
Inducible Clindamycin Resistance(Neg/Pos)	NEG	NEG	NEG	NEG
Erythromycin (<= 0.25)	<= 0.25	S	S	S
Telithromycin (<= 0.25)	<= 0.25	S	S	S
Clindamycin (<= 0.25)	<= 0.25	S	S	S
Quinupristin/Dalfopristin (<= 0.25)	<= 0.25	S	S	S
Linezolid (<= 0.5)	2	S	S	S
Teicoplanin (<= 0.5)	<= 0.5	S	S	S
Vancomycin (<= 0.5)	1	S	S	S
Tetracycline (<= 1)	>= 16	S	S	R
Tigecycline (<= 0.12)	<= 0.12	S	S	S
Nitrofurantoin (<= 16)	<= 16	S	S	S
Fusidic Acid (<= 0.5)	>= 32	S	S	R
Mupirocin (<= 2)	>= 512	S	S	R
Rifampicin (<= 0.5)	1	S	S	S
Trimethoprim/Sulfamethoxazole (<= 10)	<= 10	S	S	S

Abbreviations: R, resistant; I, intermediate; S, susceptible; Neg, Negative; Pos, Positive; NR, No reaction; MIC, Minimal Inhibitory Concentration.

was investigated by means of penicillin binding protein 2 (*mec*A, Accession No. JN108029) of *S. aureus* and GeneDoc program. Each showed 99.7% homology in comparison to *mec*A (Accession No. JN108029) gene on Genebank.

DISCUSSION

As annual medical expense is increasing due to infections in hospitals, medical centers are operating a hospital infection management center, however, there is insufficient research on the degree of bacterial infection with the targets of medical care center workers. Recently, with the rise of hospital infection, bacterial infection on clothes, hands and in the nasal cavities of hospital workers such as doctors, specialists and nurses were investigated. The results found CNS, *S. aureus*, and others, with MRSA being isolated.[1,21] Healthcare workers' mobile phones are potential bacterial transmission factor between patients and healthcare workers in hospitals. To identify nosocomial infection bacteria,

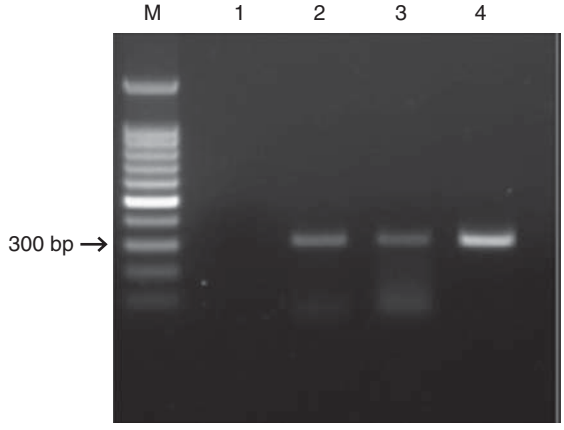

Figure 1. Polymerase chain reaction (PCR)-based amplification of *mec*A gene with DNA extracts of *S. aureus* ATCC29213, MRSA ATCC 33593, and MRSA 3rd and 9th strains isolated from dental hospital workers. The products were subjected to 1% agarose gel electrophoresis to confirm the expected length of the gene fragment (approximately 264 bp). Lane 1, PCR product of negative control; lane 2, PCR product of positive control; lane 3, 4, PCR product of MRSA 3rd and 9th strains isolated from dental hospital workers.

they used oxacillin disc diffusion test for MRSA and a double-disc synergy test for the expanded-spectrum beta-lactamase producing *Escherichia coli*.[22] Clinical professionals wear uniforms for protection from environmental contamination. MRSA and *Acinetobacter baumannii* were isolated from the uniforms of healthcare workers.[19] Patients in intensive care units have low immune systems, and their healthcare workers' hands and rings were found to be contaminated with *Staphylococci spp.*, *Klebsiella spp.*, *Enterobacter spp.*, *Escherichia coli*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Candida spp.*, *Rhodotorula spp.*, and *Aspergillus spp.*[20] In addition, many study groups isolated nosocomial infection bacteria from healthcare workers' gowns, gloves, shirt sleeves, and ties.[23,24,25] Moreover, concern about the infection of workers in dental hospitals, lead to infectious hepatitis B virus and *Mycobacterium tuberculosis* being investigated and found on patients visiting the dental hospital. When the route of the spread of infection to dental hospital workers was investigated it revealed no conclusive information.[8] As a result of the investigation on the distribution of bacteria in dental unit water systems and human saliva, *Proteobacteria, Firmicutes, Bacteroides, and Alphaproteobacteria* were isolated from both, but there was no evidence that these bacteria were the cause of bacterial contamination from dental treatments.[6]

So far in our country, many studies on environmental hygiene of dental hospitals and whether a dental hospital patient contains any infectious disease have been conducted. But there is no research on the degree of bacterial infection, with the targets of dental hospital workers. We intended to isolate pathogenic bacteria from hands and the nasal cavity of dental hospital workers. As a result of isolation of pathogenic bacteria from dental hospital workers' hands, *S. aureus*,[3] *S. capitis*,[6] *S. epidermidis*,[19] *S. hominis*,[20] *S. warneri*,[13] *S. xylosus*,[21] *S. leutus*,[9] *Micrococcus spp.*,[20] *S. marcescens*,[17] *P. aeruginosa*,[9] *K. pneumonia*,[17] *P. pneumotropica*,[12] and *S. cohnii*[12] were isolated, and as a result of investigation on *S. aureus*[3] strains for MRSA, two strains were found to be MRSA. When we isolated pathogenic bacteria from the nasal cavity of dental hospital workers, *S. aureus*,[11] *S. capitis*,[12] *S. epidermidis*,[18] *S. hominis*,[6] *S. warneri*,[9] *S. xylosus*,[2] and *S. lugdunensis*[12] was isolated, and when we investigated *S. aureus*[11] strains for MRSA, two strains were found to be MRSA. As a result of tracing MRSA from two strains, isolated from hands and nasal cavity, the MRSA detected was from the same person. The antimicrobial susceptibility test was conducted on *S. aureus* of the two strains isolated to investigate the degree of resistance and susceptibility against antibiotics other than Oxacillin. PCR was used to find out whether penicillin binding protein 2 (*mec* A) gene exists or not. Consequently, both of the strains were found to be MRSA. *S. aureus* isolated not only from patients but also from medical staff, various medical devices and the wider environment. In cases of patients with a weak immune system infection can develop into pneumonia and sepsis.[8] As MRSAs, which achieved a resistant gene among *S. aureus*, increase, the use of beta-lactam type antimicrobial is being limited.[14,26] For this reason, the usage of Vancomycin, the strongest antibiotic of gram positive bacteria tends to increase, and as Vancomycin Resistant *S. aureus* (VRSA) has been also reported, we conducted an antimicrobial susceptibility test to investigate VRSA, but it was not isolated (Table 1). Among the gram negative bacillus isolated from hands, *S. marcescens*,[17], *P. aeruginosa*,[9] *P. pneumotropica*,[12] and *K. pneumonia*[17] are opportunistic infection bacteria, known to cause nosocomial infection.[4] More studies on the spread pattern of these bacteria is necessary, but hospital infection bacteria isolated from hands of dental hospital workers are considered noticeable. Surgical operations are also conducted in dental hospitals, and implant operations are widely generalized to supplement tooth loss.[27] From dental peri-implantitis and chronic periodontitis, anaerobic gram-negative bacilli (most isolated), gram-negative cocci, and spirochaetes are isolated, and from orthopaedic infections, *S. aureus* and coagulase-negative *Staphylococci* are isolated, causing problems.[27] Patients can have a disease because of re-infection of microflora, which they themselves have. But there is also a possibility that bacteria that dental hospital workers, such as dentists and dental hygienists retain, are spread to patients.

In cases where seniors, or patients with a low immune system visit a dental hospital to undergo oral cavity operations, implants and surgical removal of a malignant tumor, they can be infected by the bacteria present on dental hospital workers. Therefore, it is expected that this investigation on bacteria from the hands and the nasal cavities of dental hospital workers, where infection can occur easily, will provide basic data for dental hospital infection management.

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