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Identification and Characterization of *Staphylococcus hyicus* Isolated from Bacterial Biofilms on Urinary Catheters

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Staphylococcus hyicus **which is an important animal pathogen, was recovered from urinary catheter of patients undergoing long-term bladder catheterization. The antibiotic sensitivity tests results showed that the isolates were susceptible to ciprofloxacin, cephalothecin, amikacin, gentamicin and ampicillin, but they were resistant to streptomycin, erythromycin and nalidexic acid. The isolates showed variable adherent capabilities to uroepithelial cells. The plasmid profile of the isolates gave two to three bands. The plasmid curing experiments indicated possible correlation between plasmid contents of *Staph. hyicus* and its adhering ability to uroepithelial cells.**

Key words: *Staphylococcus hyicus*, antibiotic resistance, urinary catheter, plasmid, adherence.

Staphyllococcus genus is one of the genera of the family micrococaceae, the bacterial species of this genus are usually found on mucous membranes and skin of humans and other warm blooded animals. Three species of this genus are important pathogens in human namely, *Staph. aureus, Staph. epidermidis* and *Staph. saprophyticus*. Whereas, *Staph. hyicus* which has been isolated from vertebrates within order artiodactyla, is found as an important animal pathogen, but is not considered as human pathgen[1]. This bacterium was found to cause bovine mastitis , exudative epidermitis in young pig, skin lesions of chicken and turkeys, and isolated from infected white tailed deer[2,3].

Indwelling medical devices (IMD) and inert surfaces have become a significant part of medical practice, but unfortunately it was found that microorganism were capable of forming attached sessile communities called biofilms on these surfaces[4]. In this respect , urinary catheters are associated with biofilm infection, it is proposed that urinary tract infection (UTI) usually follows formation of biofilm on both the internal and external catheter surface[5]. The biofilm protects microorganisms from both antibacterials and the host immune response[6,7]. *Esherichia coli, Proteus mirabilis, Staph. aureus , Staph. saprophyticus* and *Candida* species remain the most infecting

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microorganisms of urinary system[8,9]. In this investigation we report isolation and characterization of *Staph. hyicus* from biofilm of urinary catheter of patients undergoing long-term bladder catheterization .

MATERIAL AND METHODS

Isolation and characterization of bacteria from catheters

Urinary catheters from patients being cared for in Baghdad's hospitals were collected during 2006-2007. Sections three centimeters from catheter tips were cut and suspended in quarter strength ringer's solution in sterile test tubes. *Staphylococci* identification was determined following the standard methods [10]. The api Staph (bioMerieux) test was used to ascertain the identification of staphylococcal isolates as *Staphylococcus hyicus.*

Antibiotic sensitivity

Antibiotic sensitivity patterns of *Staph. hyicus* isolates were ascertained with discs assays on Mueller Hinton agar plates.

Adhesion test

Isolates of *Staph. hyicus* were selected on the basis of their multiple antibiotics resistance, each isolate was cultured in 10 ml nutrient broth, incubated overnight at 37° C and prepared for adhesion test according to the procedure reported by Iwahi *et al.,* 1982[11].

Plasmid curing

Acridine orange (AO) was used as curing agent for plasmid DNA, the experiments were done as described by Miller, 1972[12]. Plasmid DNA was isolated by modified alkaline method, and bands were detected on agarose gel electrophoresis[13,14].

RESULTS AND DISCUSSION

121 Sections from thirty seven urinary catheters were collected from patients being cared for in hospitals during 2006-2007. The microbiological tests gave 14 positive cultures which were *Staphylococcus sp*., two of these cultures were identified as *Staph. hyicus*. Six well isolated colonies from each culture were subjected

Table 1. Antibiotic resistance patterns and adherence capability to uroepithelial cells of *S.hyicus* isolates. Numbers between brackets indicate the results of AO cured bacterial derivatives

S. hyicus culture	Isolate	Antibiotic resistance phenotype	Uroepithelial adherence*
A	A1	NA,E,S,C	14
	A2	NA,E,S	10
	A3	NA,E,S,C	8
	A4	NA,E,S,TE	15
	A5	NA,E,S	9
	A6	NA,E,S,C	11
B	B1	NA,E,S,C,TE	21
	B2	NA,E,S,C,TE	24
	B3	NA,E,S,TE	29 (5)
	B4	NA,E,C,S,TE	34 (7)
	B5	NA,E,S,C,TE	28
	B6	NA,E,S,C,TE	31 (6)

Nalidixic acid (NA), Erythromycin (E), Streptomycin (S), Chloramphenicol (C), Tetracycline (TE), * (mean number of adherent bacterial cells/ uroepithelial cell).

resistance determinants between strains and species. Clinically gene transfer by plasmids is the most common mechanism of transferring resistance in bacteria[15]. The contamination of urinary catheter with *Staph. hyicus* will complicate the problem of spreading of antibiotic resistance . Moreover , it is important to consider the observation reported in this study that *Staph. hyicus* ability to adhere to human uroepithelial cells. It has been indicated that bacterial adherence is of great importance for pathogenesis of UTI [16].

CONCLUSIONS

Staph. hyicus was isolated from urinary catheter indicating the risk of transfer of bacteria from animals to human. All of *Staph. hyicus* isolates were resistant to streptomycin and erythromycin and have shown variable resistance to tetracycline and chloramphenicol. Acridine orange showed a powerful activity as curing agent for elimination of plasmid(s) responsible for erythromycin resistance. The reported results suggest the correlation between adhesion ability and plasmid contents of *Staph.hyicus*.

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