VANCOMYCIN RESISTANCE AND BIOFILM FORMATION AMONG METHICILLIN RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM CONJUNCTIVITIS

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ABSTRACT

Fifteen isolates of methicillin resistant coagulase negative staphylococci (MRCONS) recovered from fifteen cases of bacterial conjunctivitis were tested for their susceptibilities to ten antibiotics, by disc diffusion method, the extent of their susceptibilities to vancomycin by agar dilution method and, their capability for producing biofilm. Multiple antibiotic resistance was clearly recognized among all MRCONS and one strain was found resistant to all antibiotics. Six out of the 15 strains (40%) exhibited intermediate resistance to vancomycin (8-16 µg/ml) and one isolate (6.7%) recovered from adult age group was fully resistant to vancomycin (32 µg/ml). Biofilm production was expressed by 11 out of the 15 strains. These constituted seven isolates of VRCONS (100%) and four isolates out of MRCONS (50%). The association between biofilm formation and antibiotic resistance reflects clinical significance of these isolates and the need for determination of antibiotic susceptibility directly against biofilm-associated organisms.

INTRODUCTION

Over the past 20 years there has been an increase in the documentation of ocular infection caused by coagulase negative staphylococci. CONS have been reported to be the most frequent isolated pathogens from patients with acute bacterial conjunctivitis, keratitis, and post operative endophthalmitis[1-5]. The tendency of CONS to develop biofilm is widely considered to be an important determinant of prosthetic device related infection[6-8], and is also associated with virulence in the absence of prosthetic material in animal model[9,10], The process of biofilm formation is particularly relevant for clinician[11] because biofilm associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents[12,13]. NNIS report[14] in 2000 indicated that 75% of CONS from ICU were resistant to methicillin. Moreover, staphylococci have continued to mutate and have developed reduced susceptibility to glycopeptides[15]. Dunn, *et al.*[16] has reported that 50% of CONS exhibited either moderate susceptibility or resistance to vancomycin. Therefore, the aim of the present study is to evaluate antibiotic susceptibility of MRCONS recovered from bacterial conjunctivitis, to detect the extent of their susceptibility to vancomycin and to demonstrate their abilities to produce biofilm.

MATERIALS AND METHODS

Bacterial strains

A total of 15 clinically significant strains of methicillin resistant cogaluse negative staphylococci (MRCONS) recovered from patients with bacterial conjunctivitis[5] were encountered in the present study. They were the sole isolates recovered on mannitol salt agar supplemented with 10% methicillin and were negative for coagulase and DNase tests[17].These MRCONS isolates were obtained from Al-Hadithi *et al.5;* they included one isolate from neonates, five isolates from children> I month - 14 yrs. and nine isolates from adults[5].

Antibiotic susceptibility testing

Susceptibility to ten antibiotics was determined by disc agar diffusion (Kirby-Baur method)[18]. The following antibiotics were tested: Penicillin G (10IU), Cloxacillin (5µg) Tetracycline (30 IU), Tobramycin (10µg), Gentamicin (10µg), Vancomycin (30µg), Chloramphenicol (30µg) and Cephalothin (CF 30µg). Vancomycin resistance determined by disc agar diffusion was confirmed by agar dilution method (CLSI formely NNCI)[19]. n brief, from overnight cultures of MRCONS, 2-5 pure colonies were grown in 5ml Muller-Hinton broth supplemented with 2% NaCl, and incubated at 37ºC for 4-6h. Aliquotes of 0.1ml were spread onto Muller Hinton agar plates supplemented

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with 4µg/ml, 8-16µg/ml and 32µg/ml vancomycin. Plates were incubated at 37ºC for 48h. Cell growth was inspected at 24h. and 48h. The strain was judged to be vancomycin resistant if confluent cell growth was seen within 24h. and a possible heteroresistant strains of a cultivable number of colonies was appeared within 48h.

Detection of biofilm production

The method of Christensen, *et al.*[20] (1982) was adopted. In brief, twenty colonies of each MRCONS strain were transferred into sterile plastic tubes containing 5ml trypticase soy broth and incubated at 37C for 24h. Contents of the tubes were discarded then empty tubes were stained with safranin. Safranin was poured off and tubes were left to dry. Biofilm production was reported to occur when inner sides of the tubes were stained. Formation of a circle at liquid interface was reported as negative result.

RESULTS

Antibiotic susceptibility testing of 15 MRCONS are given in (Fig-1). All isolates were resistant to penicillin (100%) followed by tetracycline and cloxacillin (88.7% each). Only one isolate was resistant to vancomycin (30µg). Screening isolates for growth on vancomycin containing media has revealed that 6 out of 15 isolates (40%) exhibited intermediate resistance to vancomycin (VICONS). Only one isolate (6.7%) was found completely resistant to vancomycin (VRCONS) which was recovered from adult age group (Table-1). Four strains of the 6 VICONS which were also recovered from adult age group were found heteroresistant.

Table-2 clarifies distribution of biofilm producing strains among age groups which was expressed by 11 out of the 15 strains.

All vancomycin resistant strains (7 isolates: 2 VICONS+4 H-VICONS+1 VRCONS) were found to produce biofilm (100%). Also one isolate from each neonates and children in addition to 2 isolates from adult age group were capable of producing biofilm of the MRCONS (50%) were capable of producing biofilm.

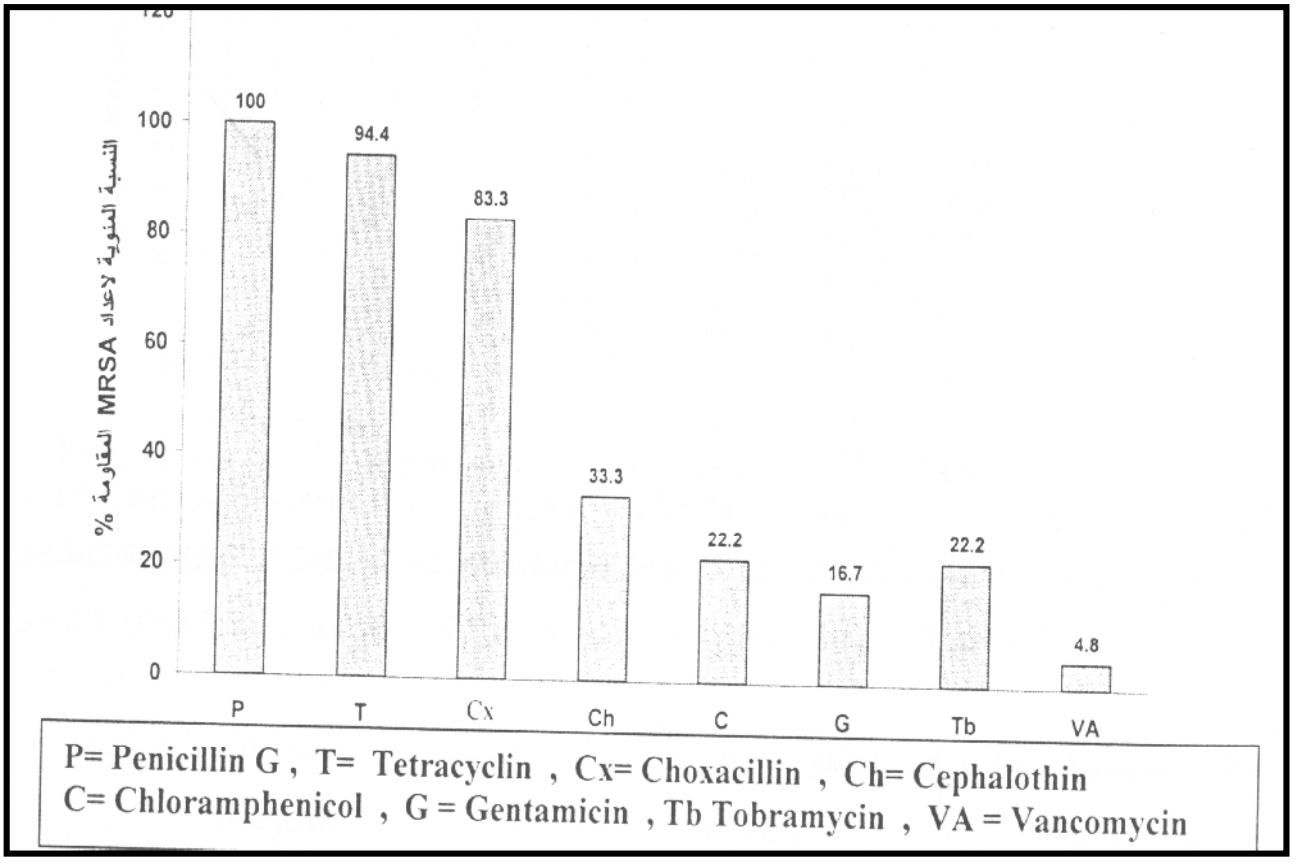

Fig 1. ***Percentage resistance of 15 MRCONS to common antibiotic.***

Table 1. ***Distribution of vancomycin resistant patterns among MRCONS in various age groups.***

Age group	MRCONS	VICONS* 8-16mg/L	H-VICONS** 8mg/L	VRCONS 32mg/L
Neonates (Less than 30 d.)	1	-	-	-
Children >month 14yr.)	5	2(40)	-	-
Adults (15-77 yrs)	9	-	4(44.4)	1(11.1)
Total	15	2(13.3)	4(26.7)	1(6.7)

* VICON= Vancomycin Intermediate resistant Coagulase Negative staphylococci
** H= Heteroresistant

Table 2. ***Frequency of slime production among MRCON and VRCONS in various age groups.***

Age group	MRCONS	Slime producers No. (%)	VRCONS (%)	Slime producers No. (%)
Neonates (Less than 30 d.)	1	1(100)	-	-
Children >month 14yr.)	3	1(33.3)	2	2(100)
Adults (15-77 yrs)	4	2(50)	5	5(100)
Total	8	4(50)	7	7(100)

DISCUSSION

Despite being important ocular pathogens, CONS have so far received little attention in ophthalmology. Because of their ubiquitous nature and relatively low virulence, staphylococci other than *S.aureus* are often reported as CONS in microbiology laboratories[21]. Multiple antibiotic resistance is clearly recognized among MRCONS isolates under this investigation. A result that support many previous studies[21,22]. Santos *et al*[23] have suggested that resistance to antimicrobial agents may emerge under different antibiotic pressures. This could explain the occurrence of variable patterns of antibiotic susceptibilities among MRCONS isolates (Fig-1) which is in accordance with finding of Pinna et al[21]. This possibly reflects skin colonization which serves as a potential reservoir for multiresistant isolates that cause infections[24,25] or as a response to prolonged treatment[26]. Detection of a strain resistant to all antibiotics tested in the present study including vancomycin, is a cause of concern as the spread of such strain in hospitals may constitute a threat for immuno-compromised[27] patients. Disc diffusion sensitivity testing using the standard 30µg vancomycin disc, frequently misclassify intermediately susceptible isolates as fully susceptible[28]. Screening isolates for growth on vancomycin containing media appears to be a sensitive way to detect even low level of vancomycin resistance[29,30]. In the present study intermediate resistance to vancomycin (8-16µg) was revealed by 6 out of 15 isolates and only one isolates was completed resistant to vancomycin (32µg) as shown in (Table-1). Four out of 6 VICONS strains recovered from adult age group gave rise to two subpopulations (one susceptible and the other resistant) which coexisted within the same culture (Table-1). This phenomenon is termed heteroresistance, and is explained by Srinivasan *etal*[28] who indicated that all cells in a culture may carry the genetic information for resistance but only small numbers may express resistance in vitro. The clinical significance of heteroresistance is not yet fully understood, however, Wong, et al[31] indicated that patients infected with heteroesistant strains did have higher mortality rates than patients infected with sensitive ones. Among MRCONS tested in the present study 11 out of 15 strains *(which constitute 50%*

MRCONS and 100% VRCONS) were found to produce slime that enables them to attach to surfaces (Table-2). The common feature of this attached growth state is that cells develop biofilm[31]. Biofilm formation, a clinically relevant process, is of great significance for public health because biofilm associated microorganisms *(sessile)* exhibit dramatically decreased susceptibility to antimicrobial agents as compared to planktonic *(suspended)* cells[12,13]. It may be a persistent source of infection, it harbors pathogenic microorganism and may allow exchange of resistant plasmids[32]. Therefore because of their increasing importance, clinically significant CONS should be identified to the species level. Moreover, susceptibility to antimicrobial agents usually relies upon response on planktonic rather than biofilm organisms. Therefore, susceptibility must be determined directly against biofilm associated microorganisms.

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