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CHARACTERISATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM SHINGLES PATIENTS

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ABSTRACT

Even after treating Shingles patients with antiviral drugs, they are found to suffer from secondary bacterial infections. With this background as a guide, we undertook an investigation to isolate the bacterial pathogens from the pus of Shingles patients. Among the isolates obtained during the one year study period, *Staphylococcus aureus* sp. was found to be multi drug resistant and hence it was chosen for the study. The antibiogram pattern of the methicillin resistant *S. aureus* was obtained, since this could serve as a tool for suggesting useful drugs.

INTRODUCTION: Antibiotic resistance has increased rapidly during the last decade, creating a serious threat to the treatment of infectious diseases. Drug resistance is one of the most serious global threats to the treatment of infectious diseases [1]. In addition to resulting in significant increases in costs and toxicity of newer drugs, antibiotic resistance is eroding our therapeutic armamentarium. Resistant strains of bacteria are continuing to increase, both in number and in variety, but not significantly different newer antibiotics are yet available.

Treatment of infections caused by these resistant bacteria has become very difficult. Since, they are resistant to many antibiotics, therapeutic options have become limited. The emergence of antibiotic resistance [2] among both pathogenic and opportunistic microbes resident in hospitals represents a serious and recurrent problem for the treatment of infections [3]. Among the several drug resistant bacteria, the methicillin resistant *Staphylococcus aureus* are common [4]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a specific strain of the *Staphylococcus aureus* bacterium which has developed antibiotic resistance to penicillins, including methicillin and other narrow-spectrum β-lactamase-resistant penicillin antibiotics [5]. MRSA was first discovered in the UK in 1961 and is now widespread, particularly in the hospital setting where it is commonly termed a superbug [6]. MRSA may also be known as oxacillin-resistant *Staphylococcus aureus* (ORSA) and multiple-resistant

Staphylococcus aureus, while non-methicillin resistant strains of *S. aureus* are sometimes called methicillin-susceptible *Staphylococcus aureus* (MSSA) if an explicit distinction must be made. Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in community and hospital settings.

The introduction of methicillin in 1959 was followed rapidly in 1961 by reports of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates. Up to 53 million people are thought to carry MRSA. Scientists estimate that around 2 billion people, some 25-30 percent of the world's population, have a form of the *Staphylococcus* bacteria [7]. Treatment of infections caused by these resistant bacteria has become very difficult, since, they are resistant to many antibiotics.

This limits therapeutic options [8]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [9]. Therefore, alternative methods of treatment are sought after.

MATERIALS AND METHODS:

Samples: The clinical study included pus samples obtained from Shingles patients suffering from symptomatic and asymptomatic bacterial infections, even after being treated with antiviral drugs from June, 2010 to June 2011. The pus specimens were transported to the bacteriology laboratory within 2 hours of collection or refrigerated for 4 hours before processing. Pus samples were brought to lab and streaked onto Nutrient agar, EMB agar, Chocolate agar, Blood and MacConkey agar with sterilized cotton swabs. Plates were incubated and observed for isolates.

Bacterial Isolates: Identification of isolates was done using standard microbiological techniques [10]. The clinical isolates obtained from the pus samples were *Esherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, S.epidermitis* and *Bacillus sp.* (**Table 1**). Among the several isolated obtained, only multidrug resistant methicillin resistant *Staphylococcus aureus* was used for the study. The isolates were maintained on Nutrient agar slants and were subcultured periodically.

TABLE 1: GROWTH OF VARIOUS ISOLATES FROM PUS IN DIFFERENT AGAR

Bacterial isolates	Nutrient agar	Blood agar	Mcconkey agar	Emb agar	Chocolate agar
S.aureus	+	+	+	-	-
S.epidermitis	+	+	-	-	+
E.coli	+	-	+	+	-
Bacillus sp	+	-	+	-	-
Pseudomonas sp	+	-	+	-	-

Detection of MRSA: All isolates in *Staphylococcus* species were tested for susceptibility to oxacillin by the agar screen method using 6µg/ml oxacillin as recommended by the NCCLS, 2002. Agar plates were incubated at 35°C and read at 24 hours and 48 hours incubation. Organisms growing on the plate were considered to be methicillin resistant.

Antimicrobial Susceptibility Testing:

Disc Diffusion Test [12]: The isolates were tested for susceptibility to antimicrobial agents on Mueller Hinton agar (Hi-Media India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards [11]. They were tested for susceptibility to the following antimicrobials: Oxacillin, Cephaloxin, Erythromycin, Chloramphenicol, Tetracyclin, Norfloxacin, Cotrimoxazole, Penicillin, Amoxicillin, Cefazolin and Furozolidine.

Antibiogram Pattern: The antibiotic profile of the several antibiotics tested against the chosen multi drug-resistant bacterial isolates is as depicted in **Table 2**. The pattern of the resistances and sensitivities of the chosen bacterial isolates are also noted.

TABLE 2-ANTIBIOGRAM PATTERN

ANTIBIOTICS	ZOI ATCC *S. aureus*	ZOI MRSA	RESPONSE
Oxacillin	22mm	13mm	Resistant
Cephaloxin	29mm	18mm	Resistant
Erythromycin	25mm	17mm	Resistant
Chloramphenicol	24mm	23mm	Sensitive
Tetracycline	26mm	23mm	Sensitive
Co-trimoxazole	25mm	19mm	Resistant
Nor-floxacin	24mm	22mm	Sensitive
Penicillin	34mm	25mm	Resistant
Amoxicillin	32mm	12mm	Resistant
Cefazolin	30mm	30mm	Sensitive
Furozolidine	20mm	20mm	Sensitive

RESULT AND DISCUSSION: Of the various bacterial isolates obtained from pus of Shingles patients, *S. aureus* isolates were resistant to several antibiotics. The antibiogram pattern was obtained for the isolated *S. aureus spp* and was compared with the ATCC control *(*ATCC 25923). The *S. aureus* strains were detected to be methicillin resistant forms (MRSA). Such methicillin resistant *S. aureus* were found to be resistant to antibiotics as oxacillin, cephaloxin and many more as depicted in table (Table 2).

Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented[13].The emergence of antibiotic resistance[2] among both pathogenic and opportunistic microbes resident in hospitals represents a serious and recurrent problem for the treatment of infections[3]. Emergence of such resistance raises question about the future of these drugs in chemotherapy, as the transmission of such resistance plasmid to other bacteria will help in the fast dissemination of resistance genes [14].

This emergence of drug resistance may be due to indiscriminate use of antibiotics and easy access and availability of various drugs in the market without prescription. Clinicians, pharmacists and general public at large must be taken into confidence to spread awareness of this problem in order to combat the spread of the resistant strains. Hence, it is clear from the study that the secondary infections among Shingles patients are definitely a life threatening problem if it is not properly taken care of.

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