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ORIGINAL ARTICLE

Multidrug Resistance and Phage Pattern of Staphylococcus aureus in Pyoderma Cases

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Abstract:

Background: Pyoderma is common in India and other tropical countries. *Staphylococcus aureus* is the commonest causative agent of pyoderma. *Aims and Objectives*: To know the antibiotic susceptibility and bacteriophage pattern of *Staphylococcus aureus* isolated from pyoderma infection. *Materials and Methods*: One hundred clinically diagnosed pyoderma cases were investigated bacteriologically. A total of 59 isolates of *S. aureus* were subjected to antibiotic susceptibility testing by Kirby Bauer's disk diffusion method and phage typing by routine test dilution X 100 bacteriophages.

Results: Most of the strains were resistant to penicillin, ampicillin and were susceptible to gentamicin, streptomycin and erythromycin. Multidrug resistance was also high among these strains. Regarding the phage types, Phage type 52 (15 strains), 96 (8 strains) and 71(16 strains) were predominant among the typed strains (55.95%) of *S. aureus*. The most common group was mixed phage group (17%) followed by phage group I (13.55%). *Conclusion*: Knowledge of antibiotic susceptibility pattern is essential to give proper antibiotic therapy and avoid unnecessary medication with non-effective drugs, which may increase resistance. Gentamicin, streptomycin and erythromycin are the drugs of choice in that order. Association of phage typing and antibiotic sensitivity of *S. aureus* showed the predominance of phage group III with greater frequency of penicillin resistance.

Key Words: pyoderma, *Staphylococcus aureus*, multidrug resistance, phage typing

Introduction:

Pyoderma is a common bacterial skin infection accounting for nearly 25% of patients attending dermatology OPD in India and other tropical countries [1]. *Staphylococcus aureus* is a versatile organism and is the commonest causative agent of pyoderma [2, 3]. Emergence of multidrug resistant *Staphylococcus aureus* has led to treatment failure in pyodermal infection; hence a detailed knowledge of antibiotic susceptibility pattern should be available for treatment. Certain phage types of *S. aureus* are more commonly associated with pyoderma and vary at different places [4]. The present study was undertaken to determine antibiotic susceptibility, prevalent phage type pattern of *S. aureus* in this area, to select the antibiotics to treat the patient and for epidemiological study.

Materials and methods:

A total of 100 clinically diagnosed cases of pyoderma who had not taken any systemic antibiotics or applied any topical preparations for the past one month, were included in this study. The present study was conducted at department of microbiology, Dr. V. M. Govt. Medical College, Solapur, (Maharashtra), India and approved by ethical clearance committee of our institute. After taking informed consent pus samples were collected under aseptic precautions. Samples were processed for Gram's stain and inoculated on blood agar and MacConkey's agar. The characteristic colonies were identified by standard methods. Out of 100 clinical specimens a total 59 strains were isolated and identified as *S. aureus.* These strains were subjected for antibiotic susceptibility testing and phage typing.

Antibiotic sensitivity of each *S. aureus* strain was determined by Kirby Bauer's disk diffusion method on Muller-Hinton agar, using the following antibiotic discs: Penicillin, ampicillin, cotrimoxazole, chloramphenicol, cefazolin, tetracycline, neomycin, streptomycin, erythromycin and gentamicin. In each test, *S. aureus* ATCC 25923 strain was used as control [5]. All the isolated *S. aureus* strains were sent for bacteriophage typing at the Staphylococcal phage typing Center, Dept. of Microbiology, Maulana Azad Medical College, New Delhi. The typing was done employing 23 sets of phages, in a routine test dilution (RTD) X 100.

Results:

A total of 59 strains of *S. aureus* were isolated from 100 pus samples of pyoderma cases. Of the 59 strains, 58 (98.30%) were resistant to penicillin and 48 (81.35%) were resistant to ampicillin (Table-1).

Table 1: Antimicrobial resistance pattern of 59 *Staphylococcus aureus* strains isolated from pyoderma.

Antibiotics	Susceptible (%)	Resistance (%)
Penicillin (10 units/disc)	01 (01.7)	58 (98.3)
Ampicillin (10 µg/disc),	11 (18 .7)	48 (81.3)
Cotrimoxazole (10 µg/disc)	18 (30.5)	41 (69.5)
Chloramphenicol (30 µg/disc)	25 (42.4)	34 (57.6)
Cefazolin (30 µg/disc)	32 (54.3)	27 (45.7)
Tetracycline (30 µg/disc)	36 (61)	23 (39)
Neomycin (30 µg/disc)	37 (62.7)	22 (37.3)
Streptomycin (10 µg/disc)	42 (71.2)	17 (28.8)
Erythromycin (15 µg/disc)	31 (69.5)	28 (30.5)
Gentamicin (15 µg/disc)	54 (91.5)	05 (08.5)

Table 2: Distribution of phage patterns of *Staphylococcus aureus* among the pyoderma cases.

Phage group	Number of Isolates (%)	Phage type (number isolates) at R.T.D. X 100
I	8 (13.55)	52 (4), 52A (1), 52/80 (1), 29/52/80 (1), 79 (1).
II	7 (11.80)	71 (4), 3A (1), 3C (1), 3C/71 (1).
III	6 (10.15)	75 (2), 42E (1), 83A/85 (1), 47/54/75 (1), 42E/83A/85 (1).
Miscellaneous	2 (03.45)	96 (2).
Mixed	10 (17.00)	52/96 (2), 52/77/96 (1), 29/52/79/81 (1), 42E/81 (1),
		29/52/81 (1), 52/42E/96 (1), 29/52/80/83A /85 (1),
		52/53/71/96 (1), 52/52A/81/83/96 (1),
Total typable	33 (55.95)	

There was not a single strain which was resistant to less than two antibiotics out of ten antibiotics studied. Only 5 (08.47%) strains were resistant to any two antibiotics. Remaining 54 strains were either resistant to any three (16.95%), four (20.34%), five (22.04%) or more number (32.20%) of antibiotics. In our study, 44 (74.58%) strains were resistant to more than three antibiotics.

Out of 59 isolates of *S. aureus*, 33 (55.95%) strains were typable and remaining 26 (44.05%) were non-typable. The most common group was mixed phage group (17%) followed by phage group I (13.55%). Phage type 52 (15 strains), 96 (8 strains) and 71 (16 strains) were predominant among the typed strains of *S. aureus* (Table-2).

Discussion:

Among the 100 cases of pyoderma, *S. aureus* has been responsible for 59 cases. These isolates have shown resistance to penicillin, ampicillin, as well as tetracycline. The antimicrobial susceptibility pattern has revealed that gentamicin has been the most effective drug against *S. aureus* followed by streptomycin and erythromycin. Other workers have also reported the same observations [6-7]. Bhat KG et al [2] has reported that gentamicin and cloxacillin are the most effective antibiotics against *S. aureus* causing pyodermal infection, while Ramani TV et al [3] has reported that *S. aureus* strains have been more susceptible to erythromycin [6] and kanamycin. Now penicillin resistance is a well known phenomenon. The highest resistance has been observed to penicillin (98.30%) followed by ampicillin (81.35%). Same antibiotic resistance pattern has been reported in many studies [1-2, 6-9].

Multidrug resistant (MDR) *S. aureus* among the pyoderma cases are very high. Majority of

MDR strains are isolated from superficial infections [10]. There are many reports from our country regarding the increase in resistance in *S. aureus*. Multiple drug resistance in *S. aureus* of 56.5% has been reported by Kar PK et al [9]. The mechanisms of multidrug resistance in *S. aureus* are drug inactivation or modification due enzymatic deactivation and alteration of target by the bacteria. The production of penicillinase, phosphotransferase, acetyltransferase and adenyltransferase enzymes are capable of inactivating the antibiotics [10]. These characters are probably plasmid borne. *S. aureus* may acquire vancomycin resistance gene (plasmid) through conjugation from enterococci [11]. There is a possibility of a circulating pool of genes that transcend species barrier and determine antibiotic resistance [12]. So, the strains isolated from clinical specimens should be tested at least for penicillinase production. Penicillinase producing *S. aureus* infections can be treated with penicillinase resistant penicillins [2].

The modes of acquisition of antibiotic resistance by *S. aureus* and possible ecological effects have also been reviewed [10]. In India multiple drug resistance is probably due to indiscriminate use of antibiotics (isoxazoyl penicillin), inadequate investigation facilities and lack of proper antibiotic policy in clinical practice. Many studies have demonstrated a decrease in multiresistance over a period, by controlling these three factors that seem to have made a major dent on the problem [10, 12].

Treatment of staphylococcal infections is becoming increasingly difficult in view of the widespread presence of MDR strains. [13]. Bacteriophages have been found to be a significant vector of antibiotic-resistance. Strains lysogenic for phage 188 acquire both chromosome and plasmid-determined resistance markers and the lysis of the donor cells is apparently required for the acquisition of resistance determinants [14].

The bacteriophage typing is used as a precise identification method of *S. aureus* and has provided valuable information in epidemiological studies of infection in the community [4]. The bacteriophage typing of *S. aureus* strains revealed that, the most common pattern is mixed (17%), as these particular organisms lysed by phages belonging to more than one group. Phage group I has been common in isolates from hospital acquired and endemeic infections, is the major group (13.55%) in our study. Antibiotic resistant strains usually belong to phage group III. In our study, 40.20% penicillin resistant strains belonged to phage group III. Similar findings have been noted with tetracycline and streptomycin [3]. The strains probably harbour plasmid(s) coding for resistance to penicillin and tetracycline. There is a simultaneous loss of a prophage carried in these strains [12]. Most of the workers have reported predominance of phage group III and II of *S. aureus* in pyoderma cases in different studies in India and the available reports from abroad. But in our study only 10.15% strains have belonged to phage group III and 11.80% to groupII. There have been only 02 (3.45%) strains in miscellaneous group but this group is infrequent in other studies. The

incidence of phage groups I, II, III and IV, miscellaneous, mixed and non-typable has been shown to varied from 1.2 to 24.9, 02 to 17.8, and 12.4 to 30.00 to 2.6, 00 to 10.4, 4.1 to 25.2 and 29.4 to 56.3 percents respectively. Such a wide variation in incidence has been observed among various reports published at different places and at different time by many workers (3- 4, 8). Phage type 52 (15 strains) has been very common followed by 96 (8 strains) and 71 (6 strains) in our study.

Non-typablility of *S. aureus* strains is a major problem employing the available sets of bacteriophages in India and other developing countries [5]. In our study 26 (44.05%) strains have been non-typable. This non-typability can be reduced by using routine test dilution strength and heat shock treatment before phage typing of *S. aureus.* Bacterium can alter its phage restricting activity, which is unlikely to occur at 37^0C [15].

The treatment of bacterial infections with bacteriophages is termed phage therapy [16]. The experimental phage therapy in animals and humans suggests that phage therapy could be an alternative to antibiotics for the treatment of infections caused by MDR strains. Recently Ryszard et al [17] have described a case of the successful eradication of MRSA carrier status in a healthcare worker. Moreover, they have reported that, a significantly lower cost of phage therapy constitutes an important additional argument for its wider consideration in the current era of a worldwide crisis due to antibiotic resistance & the economics of healthcare. The phage K has the ability to kill a broad [16] range of newly isolated pathogenic MDR staphylococci [18]; including both human and veterinary strains and it could be valuable prophylactic agent especially in immunocompromised patients [19]. The phage may be active against systemic, local and intracellular bacterial infections. However, bacterial strains may develop resistance to a bacteriophage [13]. Nevertheless, elaborative study on phage therapy in human is needed.

The present study concludes that Phage type 52 is very common. Association of phage typing and antibiotic sensitivity of *S. aureus* has showed the predominance of mixed phage group with greater frequency of penicillin resistance. Penicillin and ampicillin are the most non-effective antibiotics against *S. aureus* causing pyoderma. The increasing drug resistance is a major obstacle in the treatment of *S. aureus* infection. So the knowledge of antibiotic susceptibility pattern is essential to give proper antibiotic therapy and to avoid unnecessary medication with non-effective drugs, which may increase the resistance. We should improve investigation facilities for the detection of MDR bacteria, stop indiscriminate use of antibiotics (isoxazoyl penicillin) and follow proper antibiotic policy in clinical practice to reduce acquired resistance by the bacteria like *S. aureus*. Gentamicin, streptomycin and erythromycin are the drugs of choice in that order. These antibiotics can be recommended for treatment of staphylococcal pyoderma, in the situations where antibiotic sensitivity testing is not possible or immediate report is not available.

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