

BACTERIOLOGICAL STUDY OF PYODERMA

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Summary

Two hundred and twenty cases of pyoderma were investigated to study the bacterial aetiology. Folliculitis formed the largest clinical group followed by infectious eczematoid dermatitis, secondary infection, impetigo, miscellaneous, furuncles and acne in descending order of frequency.

A total of 172 micro-organisms were isolated from the infected specimens examined. A single infecting organism was isolated from 65.5% and more than one type of organism from 6.3% of cases. No organism was isolated from 62 (28.2%) cases. Coagulase positive staphylococcus was isolated from 107 (48.6%), beta-haemolytic streptococcus from 18(8.2%) and both these organisms from 11(5%) cases. Coagulase positive staphylococcus (69.8%) was the predominant species followed by beta-haemolytic streptococcus (17.4%), coagulase negative staphylococcus (8.1%) and alpha-haemolytic streptococcus (4.7%).

The antibiotic resistance pattern showed maximum resistance to ampicillin, penicillin and polymyxin. None of the strains was found to be resistant to cephaloridine, neomycin and kanamycin. Low percentage of strains were found to be resistant to streptomycin, tetracycline, nitrofurazone, erythromycin, chloramphenicol and gentamycin.

Of the strains of Staph. aureus phage typed, 35 (43.7%) were not typable, 15 (18.7%) belonged to phage group III, followed by phage group II, group I and mixed group. The most prevalent phage type in group III was 42E, in group II type 3C and in the group I type 52A.

Multiple antibiotic resistant strains of Staph. aureus were found mostly in phage groups III and I and not in group II.

Pyoderma or pyogenic infection of the skin is one of the commonest conditions encountered in dermatological practice. Often, these conditions are localised to the skin and are not accompanied by constitutional symptoms. The two organisms most often considered to be aetiologic agents are staphy-

lococcus aureus and beta-haemolytic streptococcus. They have been repeatedly isolated from skin lesions of patients studied at different times and different parts of the world^{1,5}. Further studies have revealed that certain specific phage types of staph. aureus are more commonly associated with pyoderma than others^{6,7}.

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It is well known that the universal use of antibiotics has produced changes in the bacterial flora of man and enabled the development of increased resistance to antibiotics by micro-organisms

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encountered in clinical practice. Correspondingly, with the advent of wide range of topical preparations containing broad spectrum antibiotics and chemotherapeutic agents, emergence of resistant organisms had also become a problem in dermatological practice⁸.

With this background in view, the present work was undertaken to study the bacterial aetiology of pyoderma, the antibiotic resistance pattern of the isolates and to study the prevalent phage types of staph. aureus.

Material and Methods

The study group comprised 220 out-patients attending the Dermatology Department of S.V.R.R. Hospital, Tirupati during the years 1974 and 1975. Only patients who had not received any antibiotic therapy prior to their coming to the hospital were selected. Material for culture was collected from the base of the skin lesions by means of sterile swabs after cleaning the surrounding area with 70% alcohol and removal of any dry scabs, if present. In cases of pustular lesions, the material was collected with a sterile platinum loop after rupturing the pustule with a sterile needle.

The specimens were inoculated on blood agar, Mac-Conkey agar and nutrient agar plates and incubated at 37°C aerobically for 18 to 24 hours. Plates showing no growth during the first 24 hours were further incubated for another 24 hours. The organisms thus grown were identified on the basis of morphological, cultural, and biochemical characteristics as per the standard procedures⁹.

All the isolates were tested for their sensitivity to 11 antibiotics and one chemotherapeutic agent. Antibiotic sensitivity of the organisms was carried out on nutrient agar plates by filter paper disc method¹⁰.

The following are the disc concentrations used:—Penicillin 10 units; Streptomycin—10 mcg; Tetracycline—30 mcg; Chloramphenicol—30 mcg; Polymyxin—300 units; Ampicillin—10 mcg; Cephaloridine—30 mcg; Neomycin—30 mcg; Gentamycin—10 mcg; Kanamycin—10 mcg; Erythromycin—15 mcg; and Nitrofurazone—100 mcg.

Coagulase positive staphylococci were phage typed at the staphylococcal phage typing centre (ICMR), Department of Microbiology, Maulana Azad Medical College, New Delhi. The phage typing was carried out with 23 basic set of International phages¹¹. The basic set comprised:—

Group I – 29, 52, 52A, 79, 80

Group II – 3A, 3C, 55, 71

Group III – 6, 42E, 47, 53, 54, 75,
77, 83A, 84, 85.

Not allocated – 81, 94, 95, 96

Results

The number of patients with pyoderma included in the study was 220, out of which 157 (71.4%) were males and 63 (28.6%) females. The total number of infected samples examined was 220, of which 62 (28.2%) yielded no organisms. A single infecting organism was isolated from 65.5% of the specimens and more than one type of organism were isolated in 6.3% of specimens examined. A total of 172 strains of micro-organisms were isolated from the infected specimens of these patients (Table 1).

Age and sex-wise distribution of the patients included in the study is presented in Table 2. The peak incidence of pyoderma was observed in 2nd and 3rd decades of life followed by 1st decade and 4th decade. The youngest patient was 18 months and the oldest 80 years of age.

TABLE 1
Patients and infected samples included in series

No. of patients	Sex		Nature of infection			No. of Micro-organism
	Males	Females	No organism isolated	Single infection	Mixed infection	
220	157 (71.4)	63 (28.6)	62 (28.2)	144 (65.5)	14 (6.3)	172

(Figures in brackets indicate percentages)

TABLE 2
Age and Sex-wise distribution of patients

Sex	10	10-19	20-29	30-39	40-49	50-59	60	Total
Males	21	33	45	23	14	10	11	157
Females	15	27	15	4	1	—	1	63
Total	36	60	60	27	15	10	12	220

Clinico-bacteriological analysis of 220 patients examined in the present investigation is shown in Table 3. Folliculitis formed the largest clinical group with 57 patients followed by infectious eczematoid dermatitis, secondary infections, impetigo and miscellaneous group in descending order of frequency. Only 9 patients suffering from furuncles and 3 patients with acne were seen among this study group. The miscellaneous group comprised secondary pyogenic dermatoses which could not be included in the other clinical entities listed.

Of the 36 cases of impetigo, 11 (30.6%) yielded coagulase positive staphylococci whereas 8 (22.2%) yielded beta haemolytic streptococcus as a single infecting agent. Mixed infection of coagulase positive staphylococci and beta-haemolytic streptococci was found in 4 (11.1%) patients. No organism could be isolated from 9 (25%) patients (Table 3).

Similarly, coagulase positive staphylococci was found to be the predominant species in other clinical groups followed by beta-haemolytic streptococci. Coag-

ulase negative staphylococci and alpha-haemolytic streptococci were also isolated as single infecting agents from patients suffering from impetigo, folliculitis, infectious eczematoid dermatitis and secondary infections. In the mixed infection group, the predominant species were found to be coagulase positive staphylococci and beta-haemolytic streptococci (Table 3).

The overall incidence of various organisms isolated and their antibiotic resistance pattern are given in Table 4. Of the 172 isolates, coagulase positive staphylococci was the most commonly isolated organism (69.8%) followed by beta-haemolytic streptococci (17.4%). A total of 14 (8.1%) strains of coagulase negative staphylococci and 8 (4.7%) alpha-haemolytic streptococci were also isolated in the present study.

The study of overall resistance pattern revealed highest percentage of resistant strains to ampicillin (68%) followed by penicillin (62.8%), polymyxin (31.4%), streptomycin (19.2%), tetracycline (14.6%), nitrofurazone (7.6%), erythromycin (6.4%) and chloramphenicol (2.9%) in that descending

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TABLE 3

Clinico-bacteriological analysis of 220 cases of pyoderma

Clinical Distribution	Total No. of patients	No organism isolated	Single infection				Mixed infection		
			CPS	CNS	BHS	AHS	CPS and BHS	CPS and AHS	BHS and AHS
Impetigo	36	9 (25.0)	11 (30.6)	3 (8.4)	8 (22.2)	1 (2.8)	4 (11.1)	—	—
Folliculitis	57	15 (26.3)	39 (68.4)	1 (1.8)	—	1 (1.8)	1 (1.8)	—	—
Furuncles	9	2 (22.2)	6 (66.7)	—	—	—	1 (11.1)	—	—
Infections eczematoid dermatitis	48	10 (20.8)	21 (43.8)	9 (18.8)	6 (12.5)	1 (2.1)	1 (2.1)	—	—
Acne	3	1 (33.3)	2 (66.7)	—	—	—	—	—	—
Secondary infection	44	17 (38.6)	17 (38.6)	1 (2.3)	2 (4.5)	1 (2.3)	4 (9.1)	1 (2.3)	1 (2.3)
Miscellaneous	23	8 (34.8)	11 (47.8)	—	2 (8.7)	1 (4.3)	—	1 (4.3)	—
Total	220	62 (28.2)	107 (48.6)	14 (6.4)	18 (8.2)	5 (2.3)	11 (5.0)	2 (0.9)	1 (0.4)

Figures in brackets indicate percentages:

CPS : Coagulase positive staphylococci ;
BHS : Beta-haemolytic streptococci ;

CNS : Coagulase negative staphylococci
AHS : Alpha-haemolytic streptococci

order. The lowest percentage of resistant organisms was found with gentamycin (1.2%) and none was found to be resistant to cephaloridine, neomycin and kanamycin.

Analysis of the resistance pattern of coagulase positive staphylococci showed similar results with highest resistance to ampicillin followed by penicillin. None of them were found to be resistant to neomycin, kanamycin and cephaloridine. High percentage of coagulase negative staphylococci also was found to be resistant to ampicillin followed by penicillin, streptomycin, tetracycline, polymyxin, erythromycin, chloramphenicol and nitrofurazone. All the strains were sensitive to cephaloridine, neomycin, gentamycin and kanamycin. Of the beta-haemolytic streptococci, only 4 strains were resistant to streptomycin and 2 to nitrofurazone and all of them

were sensitive to other antibiotics tested. Alpha-haemolytic streptococci showed resistance to penicillin (25%) tetracycline (12.5%) erythromycin (25%) and nitrofurazone (25%) (Table 4).

Of the 120 coagulase positive staphylococci, 80 strains could be phage typed and the phage pattern is shown in Table 5. Out of 80 strains, 35 (43.7%) were typable. Of the typable strains, 15 (18.7%) belonged to phage group III and 9 (11.3%) to phage group II. 7 strains (8.7%) belonged to phage group I and 4 to mixed group. The most common phage type in group III was 42E with 3 strains. In group II, the commonest type was 3C with 6 strains and in group I, type 52A with 3 strains.

Table 6 shows correlation between phage groups of coagulase positive staphylococci and their resistance

TABLE 4

Resistance pattern of the isolates

Organism	No. of strains	Pe	St	Te	Ch	Po	Amp	Ce	Ne	Ka	Ge	Er	Ni
Coagulase positive staphylococci	120	96 (80.0)	20 (16.7)	17 (14.2)	3 (2.5)	49 (40.8)	105 (87.5)	—	—	—	2 (1.7)	6 (5.0)	5 (5.8)
Coagulase negative staphylococci	14	10 (71.4)	9 (64.3)	7 (50.0)	2 (14.4)	5 (35.7)	12 (85.7)	—	—	—	—	3 (21.4)	2 (14.4)
Beta-haemolytic streptococci	30	—	4 (13.3)	—	—	—	—	—	—	—	—	—	2 (6.7)
Alpha-haemolytic streptococci	8	2	—	1	—	—	—	—	—	—	—	2 (25.0)	2 (25.0)
All species	172	108 (62.8)	33 (19.2)	25 (14.6)	5 (2.9)	54 (31.4)	117 (68.0)	—	—	—	2 (1.2)	11 (6.4)	13 (7.6)

Figures in Brackets indicate percentages

Pe : Penicillin St : Streptomycin Te : Tetracycline Ch : Chloramphenicol
 Po : Polymyxin Amp : Ampicillin Ge : Cephaloridine Ne : Neomycin
 Ka : Kanamycin Ge : Gentamycin Er : Erythromycin Ni : Nitrofurazone

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TABLE 5
Phage pattern of coagulase positive staphylococci

Phage group	No.	Strains Per cent	Phage type (Number of strains)
I	7	8.7	29(2); 42A (3); 80(1); 29/80(1)
II	9	11.3	3C(6); 3A(1); 71(1); 3C/55(1)
III	15	18.7	42E(3); 6; 47; 53; 83A; 85; 6/42E; 6/47; 6/75; 42E/54; 53/83A/85; 6/42E/54/77; 6/47/54/85 (1 each)
Mixed Group	4	5.0	79/3C; 29/94/96; 79/53/85; 29/79/53/85 (1 each)
Not Typable	45	56.3	—

TABLE 6
Correlation between phage groups of coagulase positive staphylococci and their resistance pattern

Phage group	No. of strains	Pe	St	Te	Ch	Po	Am	Ka	Er	Ni
I	7	6	1	2	—	6	7	—	1	1
II	9	7	1	—	—	4	8	—	—	—
III	15	13	1	3	1	6	14	—	2	—
Mixed	4	3	1	—	—	1	3	1	1	1
Not typable	45	36	10	6	—	17	37	—	1	3

Pe : Penicillin St : Streptomycin Te : Tetracycline Ch : Chloramphenicol
 Po : Polymyxin Am : Ampicillin Ka : Kanamycin Er : Erythromycin
 Ni : Nitrofurazone

pattern. Penicillin, streptomycin, ampicillin and polymyxin resistant strains were found to occur in all phage groups. Strains belonging to phage group II did not show any resistance to tetracycline, chloramphenicol, erythromycin and nitrofurazone, whereas the resistance to these drugs was found mainly in phage group I and III.

Discussion

In the present study, among the 220 patients with pyogenic skin infections, coagulase positive staphylococcus was isolated in 107 (48.6%), beta-haemolytic streptococcus in 18 (8.2%) and both organisms in 11 (5%) patients. These findings are in agreement with the reports of various other workers^{12,17}. Sheehan and Ferguson¹² and Kile et al¹³ isolated staph. aureus from 74 to 83

per cent of cases of pyoderma. Beta-haemolytic streptococcus was the second most common aetiological agent isolated from pyodermas with its incidence ranging from 2.2%¹⁴ to 18 per cent³. Lamont¹⁵ and Davis et al¹⁶ reported the occurrence of both the organisms with the percentages ranging from 2.4 to 45.5 respectively. Pasricha et al⁵ isolated staphylococcus from 136 (68%) cases, of which 131 (96.3%) were staph. aureus and 5 (3.7%) staph. albus. In our study staphylococcus was isolated from 121 cases as a single infecting agent, of which 107 were coagulase positive staphylococci and 14 coagulase negative staphylococci. In mixed infection group, coagulase positive staphylococci were also found in association with beta-haemolytic streptococci in 11 (5%) cases and alpha-haemolytic

streptococci in 2 (0.9%) cases. Beta-haemolytic streptococci were isolated from 18 (8.2%) cases as a single infecting organism whereas in 11 it was associated with coagulase positive staphylococci and in one with alpha-haemolytic streptococci. Alpha-haemolytic streptococci alone were recovered from 5 cases in the present series. It is interesting to note that the present study did not yield any gram negative bacilli as causative agents of pyoderma whereas other workers^{3,8,16}, have reported the isolation of gram negative bacilli.

Conflicting reports are available regarding the causative agents of different forms of pyoderma. According to Maclean Smith¹⁷ and Parker and Williams¹⁸, staph. aureus is the main causative organism of impetigo whereas Bigger and Hudson¹⁹ incriminated beta-haemolytic streptococcus as chief aetiologic agent of impetigo. In our study, out of 36 cases of impetigo, 11 were caused by coagulase positive staphylococci and 8 by beta-haemolytic streptococci, 3 by coagulase negative staphylococci and 1 by alpha-haemolytic streptococcus. Four cases of impetigo yielded both coagulase positive staphylococci and beta-haemolytic streptococci. In cases of folliculitis and furunculosis which are considered as primary lesions of hair follicles, staphylococcus was found to be the main causative organism. Other pyogenic dermatosis like infectious eczematoid dermatitis yielded both staphylococci and streptococci with predominance of coagulase positive staphylococcus. Flood²⁰ reported beta-haemolytic streptococcus as the chief causative agent of secondary pyogenic dermatosis while Naug and Ganguli²¹ held Staph. aureus responsible. As such, the results of our study indicate that coagulase positive staphylococci and beta-haemolytic streptococci are the two main aetiologic agents encountered in pyodermas even during the present broad spectrum era.

Out of 120 strains of coagulase positive staphylococci tested, resistance to penicillin and ampicillin was found to be high (87.5% and 80% respectively). The high incidence of penicillin resistance is comparable with the reports of previous workers^{3,22,23,24}. This high order of penicillin resistance correlates well with the degree of usage of penicillin in hospitals and general practice. The high incidence of ampicillin resistant strains encountered in our series, even though this antibiotic is not extensively used, may be explained by the fact that ampicillin is destroyed by beta-lactamase produced by penicillin resistant strains of staphylococci. The incidence of resistance to polymyxin was found to be 40.8% and this may be due to the fact that the polymyxins are large molecules and the diffusability of this drug through agar poor, thus showing very narrow inhibition zones. The percentage of resistant strains to streptomycin, tetracycline, chloramphenicol, erythromycin, gentamycin and nitrofurazone was comparatively low, running parallel to the extent of usage of these drugs in the community. All the strains of staphylococci both coagulase positive and coagulase negative were found to be sensitive to cephaloridine, neomycin and kanamycin. This finding is expected with cephaloridine since this antibiotic is active even against penicillinase producing staphylococci. As far as neomycin and kanamycin are concerned, they are closely related in their activity and are quite effective against gram positive organisms like staph. aureus and mycobacterium tuberculosis²⁵. Clinico-pathologic study by Shroff et al²⁶ also revealed that neomycin is particularly effective in cases of infective dermatosis.

Beta-haemolytic streptococci did not show any resistance to penicillin, tetracycline, chloramphenicol, polymyxin, ampicillin, cephaloridine, neomycin, kanamycin and gentamycin. Low percentage of strains was found to be

resistant to streptomycin (13.3%) and nitrofurazone (6.7%). As the number of Alpha haemolytic streptococci tested is too small, no attempt is made to discuss the resistance pattern. The overall analysis of resistance pattern of the micro-organisms isolated in our series revealed maximum resistance to ampicillin (68.0%) followed by penicillin (62.8%) and polymyxin (31.4%).

The phage typing of staph. aureus isolated in the present study revealed that majority of the strains (56.3%) were not typable with the set of phages employed. Of the typable strains, Group III is the predominant group followed by groups II, I and mixed group. Dazani et al³ and Rountree²⁷ reported a higher incidence of phage group II strains isolated from skin lesions. But our results are in agreement with that of Pasricha et al⁵ who found maximum number of strains in Group III followed by group II. Among the group III strains, the most prevalent phage type in our study is 42E where as Pasricha et al⁵ reported type 83A as the predominant type. In group II, phage type 3C accounted for 6 of the 9 strains whereas other workers^{3,5} found phage type 71 and 55 as predominant types respectively.

While correlating the phage pattern of coagulase positive staphylococci with their antibiotic resistance, it has been found that multiple antibiotic resistant strains were encountered mainly in phage group III followed by group I and not in group II. These findings are in agreement with the reports of other workers^{22,28} who also showed that resistant strains belonged to phage group III and I whereas Pasricha et al⁵ found that the majority of antibiotic resistant strains belonged to phage group III followed by phage group II and not to phage group I.

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TRUE or FALSE?

Patients with mixed connective tissue disease (MCTD) have a diagnostic serological abnormality namely that of a very high titer of hemagglutinating serum antibody to an extractable nuclear antigen (ENA).

(Answer page No. 185)