Original Article

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Microbiology Section

Species Distribution of the Aerobic Bacterial Profile in Pyoderma with Special Reference to Antibiotic Susceptibility Pattern of the Isolates at a Tertiary Care Hospital of West Bengal, India

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ABSTRACT

Introduction: Pyoderma is a common health problem characterised by pyogenic infections of the skin and its appendages. Though, easily treatable, the condition is known for its chronicity, recurrence and other complications. Therefore, timely recognition and prompt bacterial diagnosis with antimicrobial sensitivity is imperative for the effective management and treatment of pyoderma. It is a common bacterial skin infection accounting for nearly 25% of patients attending Dermatology Outpatient Department (OPD) in India and other tropical countries.

Aim: To determine the incidence of pyoderma in relation to age, sex and socio-economic status, to isolate and identify the common aerobic microbial pathogens associated with pyoderma prevalent in the community and antibiotic susceptibility pattern.

Materials and Methods: An institutional based cross-sectional observational study was conducted on 148 cases in Department of Microbiology, Rampurhat Government Medical College and Hospital, Burdwan, West Bengal, India, clinical features of suspected pyoderma for a period of 12 months from March 2021 to February 2022. Lesion swabs were collected and isolates were identified; antibiotic susceptibility testing was also performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines using antibiotic discs. Descriptive data was presented as count and percentages.

Results: Out of 148 samples collected, 144 (97.3%) were culture positive while, rest 04 (2.7%) were culture negative. Overcrowding and low socio-economic group were closely related with pyoderma patients. Primary pyoderma (72.2%) cases were detected more than the secondary (27.8%) cases. *Impetigo contagiosa* (54%) were detected more among the primary pyoderma patients. High numbers (66.7%) of pyoderma were detected among children (0-10 years). The culture positive samples were more in male patients (68.7%) than in female patients (31.3%) and mostly detected from OPD. Out of 144 isolates, 89 (61.8%) were *Staphylococcus aureus*, 23 (15.9%) isolates were Coagulase-negative Staphylococci (CoNS) and 04 (2.8%) were *Streptococcus pyogens.* Further 06 (4.2%) isolates of *Pseudomonas aeruginosa*, 10 (6.9%) isolates of *Escherichia coli* and 08 (5.5%) isolates of *Klebsiella pneumonia* were found.

Conclusion: The present study results suggest that the era of antibiotics has ushered in an unprecedented predominance of *Staphylococcal* rather than *Streptococcal* infections and other gram negative infections for pyoderma cases. Increasing incidence of methicillin, quinolones and amikacin resistance in Staphylococci and other gram negative isolates have limited treatment options. For this, a single infection like pyoderma is challenging in all the healthcare facility.

Keywords: Aerobic bacteria, Antibiotic sensitivity, Pyogenic infection, Skin lesions

INTRODUCTION

Pyoderma or pyogenic infection of the skin is defined as 'any purulent skin disease' [1]. This is one of the commonest conditions observed in dermatological practice [2]. Different studies indicate that up to 17% of the visits in the chamber of dermatologist may be for bacterial skin infections [3]. Recent estimates of the global burden of impetigo are 111 million children from developing countries [4], to 140 million people affected at any one time [5,6]. Many factors like poverty, malnutrition, overcrowding, illiteracy, customs, habits are believed to be responsible for its high incidence [7]. Climatic conditions also play a major role [8]. In hot and rainy seasons, the occurrence of pyoderma is increased [9].

Staphylococcus aureus skin and soft tissue infections include primary pyoderma (such as folliculitis, furuncles, carbuncles and impetigo) and soft tissue infections (i.e., cellulitis, erysipelas and pyomyositis). They are commonly classified according to the anatomic structure involved: infection of the epidermis: impetigo; infection of the superficial dermis: folliculitis; infection of deep dermis: furuncles, carbuncles; and infection of subcutaneous cellular tissues [10,11].

Changing trends are being noted in the aetiological aspects of primary pyoderma and the problem of emergence of drug resistance strains is increasing day by day. Methicillin-sensitive *Staphylococcus aureus* (MSSA) or Methicillin-resistant *Staphylococcus aureus* (MRSA) are most common organism usually isolated in pyoderma [12]. Many of these isolates are becoming Multidrug Resistant (MDR). All β-lactams including carbapenems and high-end cephalosporins, piperacillin, tazobactum are ineffective against MRSA [12]. Knowledge of prevalence of MRSA and their current antimicrobial profile becomes necessary in the selection of appropriate empirical treatment of these infections [12]. Studies on bacteriology of pyoderma have been done in several other regions, but none is available from our region. Common bacteria causing pyoderma are *Staphylococcus aureus*, Group *A Streptococcus,* Coagulase-negative Staphylococci (CoNS), *Pseudomonas aeruginosa*, apart from other isolates such as *Citrobacter* spp., *Escherichia coli, Klebsiella* spp, *Proteus* spp. [13,14].

Various factors like poverty, malnutrition, overcrowding, illiteracy, customs, habits and so on have been stated to be responsible for its high incidence [15]. Climatic conditions also play a role, with the hot and rainy seasons being the period of maximum occurrence [16]. Besides, patients on treatment with steroids or chemotherapeutic agents and those with pre-existing skin diseases, obesity, disorders of the immune system and diabetes are found to have bacterial skin infections more commonly [17]. A correct antimicrobial policy based on the knowledge of resistance patterns of the commonly isolated organisms is mandatory to prevent unnecessary medication and further emergence of drug-resistant organisms [18]. Hence, keeping this view in mind, the present study was designed on pyoderma to find out the incidence, causative organisms affecting and their latest antibiotic susceptibility patterns in this region.

MATERIALS AND METHODS

This was an institutional based cross-sectional observational study conducted in the Department of Microbiology, Rampurhat Government Medical College and Hospital, Rampurhat, Birbhum, West Bengal, India, for a period of 12 months from March 2021 to February 2022. The study was approved by the Institutional Ethics Committee (Memo No.IEC/2022/04/012). A total of 148 cases were selected from the different departments of Rampurhat Government Medical College and Hospital having clinical features of suspected pyoderma in the given time duration.

Inclusion criteria: Patients belonging to all age groups and either sex with any purulent skin condition presenting to the Dermatology Department (both outpatient and inpatient wings), and giving written informed consent were included in the study. For children less than 18-year-old, parental consent was taken.

Exclusion criteria: Non infected insect bites and non inflamed partly healed pyoderma lesions were excluded from the present study. Patient who had used any topical applications received systemic antibiotics, medicated soap or powder in past one week was also excluded.

Study Procedure

Sample collection: Sterile swabs were used to collect exudates aseptically from the lesions. Two samples were collected before the start of antibiotic therapy and transported to the microbiology laboratory as early as possible for culture and sensitivity examination.

Sample processing: All samples were inoculated on blood agar, MacConkey agar and Nutrient agar plates and incubated aerobically overnight at 37°C. Bacterial isolates were identified and characterised following colony morphology, gram staining, motility, biochemical reactions and detection of MRSA and antimicrobial susceptibility testing (by disc-diffusion method) of the isolates were performed following Central Laboratory Standards Institute (CLSI) guidelines [19]. CLSI performance standards for antimicrobial disc susceptibility tests, approved standards [19]. Nasal swabs cases collected from patients as follow-up who showed *S. aureus* in their pus culture.

STATISTICAL ANALYSIS

All the data was entered in a Microsoft excel spread sheet and analysed for variables. Descriptive statistics for both clinical and bacteriological characteristics was generated. Statistical comparison of categorical variables was undertaken using Chi-square test and a p-value of <0.05 was considered statistically significant.

RESULTS

A total of 148 clinically suspected pyoderma patients were included in the present study. Out of 148 patients suspected to have pyoderma infections, 144 (97.3%) patients were found to be culture positive and 4 (2.7%) patients were found to be culture negative. Out of 144 culture positive samples collected, 104 (72.2%) were of primary pyoderma while rest 40 (27.8%) were secondary pyoderma cases.

Out of total 104 patients of primary pyoderma, impetigo contagiosa were seen among 56 (54%) patients. A total of 22 (21.1%) patients showed folliculitis, 17 (16.3%) were of furunculosis, 5 (4.8%) cases were of cellulitis and 4 (3.8%) cases were of carbuncle. Out of total 144 patients whose sample showed positive culture, 96 (66.7%) patients were below 10 years of age, 21 (14.6%) patients between 11-20 years of age, 25 (17.4%) were in age group 21-50 years and only 02 (1.3%) patients were >50 years of age. Among the 144 culture positive samples, 45 (31.3%) samples from female and 99 (68.7%) samples from male patients. The present study reflects that number of high income group was 5 (3%), middle income group was 40 (27%) and Low income group was 103 (70%). According to monthly per capita income, patients were classified as per modified BG Prasad's criteria 2004 [20] [Table/Fig-1].

Parameters	n (%)
Gender	
Male	99 (68.7%)
Female	45 (31.3%)
Age (in years)	
0-15	96 (66.7%)
16-30	21 (14.6%)
31-45	25 (17.4%)
>45	02 (1.3%)
Primary pyoderma	
Impetigo contagiosa	56 (54%)
Folliculitis	22 (21.1%)
Furunculosis	17 (16.3%)
Cellulitis	5 (4.8%)
Carbuncle	4 (3.8%)
Secondary pyoderma	40 (27.8%)
Income*	
High income group	05 (3%)
Middle income group	40 (27%)
Low income group	103 (70%)

[Table/Fig-1]: Demographic data. N=144.
*For income group, N=148

Out of 144 organisms isolated, 89 (61.8%) were *Staphylococcus aureus*, 23 (15.9%) isolates were CoNS and 04 (2.8%) were *Streptococcus pyogens.* Further 06 (4.2%) isolates of *Pseudomonas aeruginosa*, 10 (6.9%) isolates of *Escherichia coli* and 08 (5.5%) isolates of *Klebsiella pneumonia* were found. Number of *Proteus mirabilis* isolates were 03 (2.1%) and *Acinetobacter* spp. isolates were 01 (0.7%) in number. Out of total 89 *Staphylococcus aureus* isolates, 9 (10.1%) isolates were MRSA and 80 (89.9%) were MSSA [Table/Fig-2].

Isolates	Total no. of isolate	Percentage (%)
Staphylococcus aureus	89	61.8%
Coagulase-negative Staphylococci (CoNS)	23	15.9%
Streptococcus pyogens	04	2.8%
Escherichia coli	10	6.9%
Pseudomonas aeruginosa	06	4.2%
Klebsiella pneumoniae	08	5.6%
Proteus mirabilis	03	2.1%
Acinetobacter spp.	01	0.7%
Total	144	100%

[Table/Fig-2]: Distribution of different isolates obtained from culture (n=144).

The antibiotic sensitivity pattern of gram positive bacteria showed vancomycin sensitivity to *S. aureus* (100%), CoNS 100%, and for *S. pyogenes* also (100%). Regarding sensitivity of cefoxitin, the percentage of sensitivity for *S. aureus* is 90% and for CoNS is 73.9%. For CoNS the sensitivity pattern of other drugs like, teicoplanin,

linezolid, amikacin and amoxyclav are 100%, 86.9%, 86.9% and 73.9%, respectively. This distribution showed in [Table/Fig-3].

Antibiotic	*Staphylococcus aureus* (n=89)	CoNS (n=23)	*S. pyogenes* (n=04)
Vancomycin	89 (100%)	23 (100%)	04 (100%)
Cefoxitin	80 (90%)	17 (73.9%)	NA
Teicoplanin	78 (87.6%)	23 (100%)	NA
Linezolid	79 (88.8%)	20 (86.9%)	04 (100%)
Amikacin	62 (69.7%)	20 (86.9%)	NA
Amoxyclav	64 (71.9%)	17 (73.9%)	NA
Chloramphenicol	NA	NA	03 (75%)
Ampicillin	NA	NA	04 (100%)
Meropenem	NA	NA	04 (100%)
Levofloxacin	NA	NA	04 (100%)

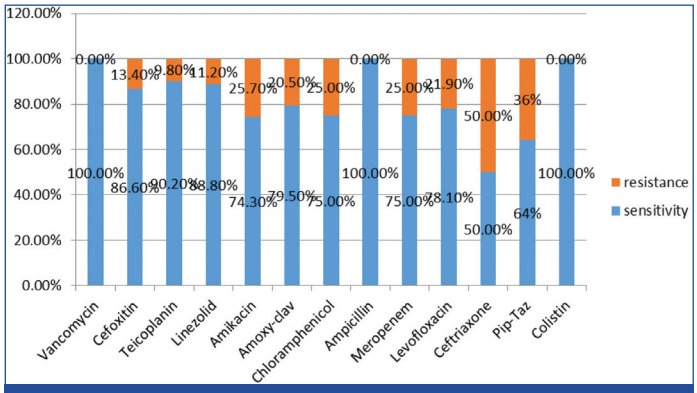
[Table/Fig-3]: Antibiotic sensitivity pattern of gram positive isolates (Total number 'n'=116).

The antibiotic sensitivity pattern of gram negative bacteria to colistin showed 100% sensitivity to *Escherichia coli, K. pneumoniae*, *P. aeruginosa*, *Acinetobacter* and *P. mirabilis*. Regarding sensitivity of amikacin, the percentage of sensitivity of individual isolates of *Escherichia coli, K. pneumoniae*, *P. aeruginosa*, *Acinetobacter* and *P. mirabilis* were 80%, 75%, 66.7%, 100% and 100%, respectively. This distribution showed in [Table/Fig-4].

Antibiotics	EC (n=10)	KP (n=08)	PA (n=06)	AC (n=01)	PM (n=03)
Amikacin	08 (80%)	06 (75%)	04 (66.7%)	1 (100%)	03 (100%)
Ceftriaxone	07 (70%)	04 (50%)	02 (33.3%)	-	01 (33.3%)
Levofloxacin	06 (60%)	07 (87.5%)	05 (83.3%)	-	03 (100%)
Piperacillin-Tazobactum	06 (60%)	05 (62.5%)	04 (66.7%)	1 (100%)	02 (66.7%)
Meropenem	06 (60%)	06 (75%)	04 (66.7%)	1 (100%)	03 (100%)
Colistin	10 (100%)	08 (100%)	06 (100%)	1 (100%)	03 (100%)

[Table/Fig-4]: Antibiotic sensitivity pattern of gram negative isolates (Total number N=28).
EC: *Escherichia coli*; KP: *Klebsiella pneumonia*; PA: *Pseudomonas aeruginosa*; AC: *Acinetobacter* spp; PM: *Proteus mirabilis*

Vancomycin was sensitive to 100% isolates, cefoxitin to 86.6%, ampicillin to 100%, teicoplanin to 90.2%, linezolid to 88.8%, Pip-Tazo to 64.3%, meropenem 75%, colistin to 100%, amoxyclav to 79.5%, levofloxacin to 78.1%, amikacin to 74.3%, and chloramphenicol to 75%, ceftriaxone to 50%. This result i.e., overall percentage of sensitivity of isolates to different antibiotics is depicted in [Table/Fig-5].



[Table/Fig-5]: Bar diagram showing overall percentage sensitivity of isolates to different antibiotics (n=144).

DISCUSSION

The present study was done over a period of 12 months, at a tertiary care hospital of West Bengal, to know the prevalence, types and bacterial aetiology of pyoderma, as well as, to know the antibiogram of the organisms causing pyoderma. In present study, total 148 samples were collected, out of which 144 (97.3%) samples were found to be culture positive. Similar study by Harshita G et al., observed on bacteriological examination of 160 samples obtained from pyogenic lesions, growth was obtained in 148 (92.5%) samples [21]. In the present study, among 144 cases shown, 104 (72.2%) cases were primary pyodermas and 40 (27.8%) cases were secondary pyodermas. The prevalence of primary pyodermas (78.9%) was higher than that of secondary pyodermas (21.1%). This is in accordance with the study by Ashokan C et al., where the incidence of primary pyoderma was 60% [22]. This present study showed that impetigo formed the largest clinical group in primary pyodermas (54%), followed by folliculitis (21%), furunculosis (16%), cellulitis (5%) and curbuncle (4%). This is in contrast to the study by Badabagni P and Malkud S, where impetigo contagiosa was the most common type of primary pyoderma 81 (27%) followed by folliculitis 66 (22%), furunculosis 30 (10%), bullous impetigo 30 (10%), ecthyma 18 (6%), sycosis barbae 15 (5%), cellulitis 9 (3%), acute paronychia 9 (3%), periporitis 6 (2%) and carbuncle 6 (2%) [23]. Other primary pyodermas noted were folliculitis, furunculosis, paronychia, cellulitis and carbuncle. Whereas, study done in Mumbai by Patil R et al., shown predominance of folliculitis and furunculosis of 58.8% and 33.3%, respectively [24].

In the present study, most of the pyodermas were observed in age group below 10 years followed by 21-50 years. Similar findings were observed by other workers such as Gandhi S et al., Mathew MS et al., who studied in paediatric patients and observed most of the pyodermas in one to four years' age group (54.2%), followed by five to eight years [16,18]. In the present study, males (68.7%) were more than the females (31.3%). Male to female ratio was 3:2. Similar findings were observed by Bhat YJ et al., where females were 29% compared to male (71%) and, Nagmoti JM et al., Kar PK et al., Malhotra SK et al., Hanif MM et al., (2012) male:female was 63:37 [25-29].

Out of 148 cases, 144 samples yielded organisms and four samples were sterile. Of the 144 positive cultures, 89 (61.8%) were *Staphylococcus aureus*, 23 (15.9%) isolates were CoNS and 4 (2.8%) were *Streptococcus pyogens.* In a study by Janardhan B et al., out of 100 cases, 88 samples yielded organisms and 12 samples were sterile [30]. Of the positive cultures, 84 yielded single organisms, whereas four showed mixed growth. *Streptococcus* was isolated in only one case as a mixed growth with *Staphylococcus aureus*. *Staphylococcus aureus* was isolated in 39 cases and CoNS in 14 cases. Parikh DA et al., however observed *Staphylococcus aureus* from 97% of cases and CoNS from 3% of cases [31].

The sensitivity of *Staphylococcus aureus* isolates to antimicrobials used showed the highest sensitivity to vancomycin (100%) followed by cefoxitin (89.9%), linezolid (88.8%), teicoplanin (87.6%). Proportionately, less sensitivity was seen to amoxicillin-clavulanic acid (71.9%) and amikacin (69.7%). Gandhi S et al., who observed *Staphylococcus aureus* isolates to be 99.35% sensitive to vancomycin and 94.35% sensitive to amoxyclav [18]. Patil R et al., in their study also reported 100% sensitivity of Staphylococcal isolates to vancomycin [24].

Regarding antibiotic sensitivity pattern, CoNS was mostly susceptible (100%) to vancomycin and teicoplanin, followed by linezolid (87%) and amikacin (87%) in present study. CoNS showed comparatively low level of sensitivity to amoxicillin-clavulanic acid (74%). A similar study by Harshita G et al., showed that among the CoNS isolates maximum sensitivity was observed to linezolid, vancomycin and amoxicillin-clavulanic acid, 100% each, followed by amikacin (85%) and maximum resistance was seen to ampicillin (75%) followed by cephalexin (60%) [21].

In the present study, only 19.5% isolates were gram negative. All the gram negative organisms were sensitive to colistin most of them were moderately sensitive to piperacillin-tazobactam, levofloxacin, meropenem and amikacin. In different studies, this is observed that, incidence of pyoderma caused by gram negative organisms is lower than the gram positive organisms and sensitivity patterns of these studies are quite similar to the present study [32]. So, on account of the high prevalence of pyoderma scenario, changing

pattern of causative microorganisms, and the indiscriminate use of antibiotics leading to altered antibiotic susceptibility pattern, there is a constant need to obtain more information about aetiological agents, predisposing factors and effective methods for control.

Limitation(s)

The present study was done at a tertiary care centre of West Bengal and highlights the clinico-epidemiological features and pattern of bacterial infections in pyodermas. A larger study involving many centres, would be having more validity and scientific rigor to support the findings and bring about changes in practices.

CONCLUSION(S)

Pyogenic skin infections are frequently encountered in day to day clinical practice. MDR has become a clinical challenge and most strains were found to be resistant to one or more antibiotics, thus, limiting treatment option. Also, if not treated promptly they are followed by various complications. MDR has resulted from indiscriminate use of antibiotics. A correct hospital antibiotic policy and the avoidance of inappropriate antimicrobial usage are mandatory to reduce the spread of antibiotic resistance in the community, also keeping newer antibiotics in reserve for use only against strains that are resistant to the common antibiotics. Hence, timely recognition, and prompt bacterial diagnosis and antibiotic susceptibility testing is very important for the management of pyoderma and also, to check the major complications.

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