Bacteriological Study of Pyogenic Skin Infection At Tertiary Care Hospital.

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Abstract

Introduction: Pyogenic skin infection (pyoderma) is the bacterial infection of skin and its appendages¹. Cutaneous bacterial infections are common clinical problems encountered in most fields of clinical medicine.² Pyoderma constitutes a major portion of patients in dermatological clinics in India.^{3,4.} Diagnosis is mainly based on clinical examination correlated with laboratory investigations

Aim & objectives: This study was conducted to study the etiology of primary and secondary pyoderma, determine the antimicrobial susceptibility pattern of the isolated bacteria, and to detect MRSA.

Material Method: Study was carried out in Department of Microbiology, over a period of one year, from 15 april 2014 to 14 april 2015, including a random group of 100 cases of clinically diagnosed pyogenic skin infections, both primary and secondary pyodermas of various age groups and of either sex visiting the Dermatology out- patient department of J.L.N. Medical College & Hospital, Ajmer. Patients on antimicrobial treatment (local or systemic) during the last 15 days were excluded from the study.

Result and Observation: Primary pyoderma was more common than secondary pyoderma. The commonest clinical type was folliculitis followed by impetigo. Infected scabies, infected eczema constituted majority of the secondary pyoderma cases. The incidence was found to be more in males than in females and adolescent age group. Out of 100 samples processed 92 yielded growth whereas 8 did not yield any growth. Out of 92 cases that yielded growth 79 cases showed only one type of growth whereas 13 cases showed mixed (two types) growth. The various organisms isolated included S.aureus, CONS, S.pyogenes, Enterococcus, E.coli, klebsiella spp, proteus spp, pseudomonas spp. S.aureus was the commonest organisms isolated. S.aureus was most sensitive to Vancomycin and Linezolid whereas least to Penicillin G. Resistance to fluoroquinolones as well as to other antibiotics tested was significantly higher in MRSA isolates than that in MSSA isolates (p<0.0001).Among gram negative bacteria , all were uniformly sensitive only to higher antibiotics like Imipenem, Piperacillin, Aztreonam.

Conclusion: 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.

Keywords: Pyoderma, folliculitis, sensitivity, S.aureus, MRSA.

I. Introduction

Pyoderma is common health problem in the low socioeconomic group,^{5,6,7} especially in young children.⁵⁻⁹ Heat, humidity specially during summers and monsoons play a major role in occurrence of the disease,^{6,7} particularly in tropical developing countries like India where it is common problem.^{10,11,12} Various predisposing factors include immunosuppression, atopic dermatitis, scabies, pediculosis, pre-existing tissue injury and inflammation.¹

Primary pyoderma are caused by direct invasion of normal skin and have a characteristic morphology while Secondary pyodermas originate in the diseased skin as a superimposed condition like in scabies, pediculosis, wounds, insects bites and eczema.¹³

S. aureus and S.pyogenes are the two important pyogenic organisms most commonly isolated from the pyoderma cases, the former being more common than the latter.^{3,4,5,7,14,15,16} Besides these, other organisms that are occasionally isolated from pyodermas are Enterococci, Pseudomonas, E.coli, Proteus, Citrobacter and Klebsiella spp.^{3,5,9,17,18}. The source of infection are either family members, school mates, hostel mates, military barracks, medical personnel, or inanimate objects like clothes, floors, walls and instruments used in hospitals. Person to person spread of organism occurs due to hospitalization of the sick person and otherwise crowded places.¹⁹

Diagnosis is mainly based on clinical examination correlated with laboratory investigations like examination of Gram stained smears of the purulent material along with culture and isolation of the causative organism and its identification by various biochemical tests.¹⁹

Treatment is given in the form of topical, oral and parenteral antibiotics. When planning therapy, local and current antimicrobial resistant patterns have to be considered.¹ Many cases do not respond to antibiotics which were previously very effective as most of the organisms isolated are found to be resistant to them.³ The injudicious and indiscriminate use of topical and systemic antibiotics has contributed to this situation,^{2,3,9,17} the wide use of antibiotics resulting in selective survival of the resistant strains.^{19,20} Multidrug resistant strains also possess the properties of transmissibility and virulence. More recently possibly as a result of introduction of newer antimicrobials and their extensive use, strains have been encountered that are resistance to greater number of antibiotics.¹⁴ Constant use of antibiotics results in survival and spread of MRSA, ESBL producers and multidrug resistant Enterococci.²¹

Aim & objectives

- 1. To study the etiology of primary and secondary pyoderma.
- 2. To determine the antimicrobial susceptibility pattern of the isolated bacteria.
- 3. To detect MRSA

II. Material And Method

Collection Of Specimen

Sterile swabs were used for aseptic collection of specimen of exudates or pus from the lesions. After cleaning the area around the lesion with 70% ethyl alcohol the specimen was collected on two sterile cotton swabs following puncturing of a fresh closed lesion with a sterile needle.

The quality control and rejection criteria for the inappropriate specimen were followed as per the standard guidelines.

At the time of enrolment, a written informed consent was obtained. A complete medical history was taken with reference to the mode of onset, duration and progress of lesion. History of any systemic illness and family history was also obtained. History of contact with an infected person and history of antibiotic intake was taken. A note about the socio-economic status of the patient was made. Thorough clinical examination was performed to find out the type of lesion and its distribution.

Transport Of Specimen

Specimens were transported and processed within 2 hours of collection by the standard microbiological technique.

Following procedures were performed:

- Gram staining
- Bacterial culture
- Identification of pathogens
- Antimicrobial susceptibility testing of pathogens

Gram Staining

Using the first swab, a smear was made on a clean glass slide and stained by Gram staining. The smear was screened for pus cells and organisms. The gram reaction, morphology, arrangement of organisms and the number of different types of organisms was noted.

Culture:

Second swab was inoculated on the following media:

- Nutrient agar
- Blood agar
- McConkey agar

The inoculated media were incubated aerobically at 37^{0} C for 24 hours. In case of no growth after 24 hours, the plates were further incubated for another 24 hours.

Identification Of Pathogens:

The pathogens were identified by standard microbiological techniques by studying their colony characteristics, morphology and biochemical reactions.

- Colony characteristics: Size, shape, elevation, margin, surface, opacity, consistency, change in the medium, pigment production, etc. were studied.
- Morphology: The portion of the colony was emulsified in a small drop of saline on a glass slide. After drying of the smear, it was fixed by using heat, quickly passing the slide 4-5 times through the flame of bunsen burner. The fixed smear was subjected to gram stain.
- Smear was covered with crystal violet and allowed it to remain on the surface without drying for 10 seconds. After rinsing the slide with water, it was covered with gram's iodine for 20 seconds and then rinsed

with water. Smear was decolorized with acetone till no violet colour washes off. The smear is counterstained with safranine for 30 seconds. The slide washed and gently blotted dry. Gram reaction, size, shape, arrangement, pleomorphism etc. were noted.

- Hanging drop preparation was also made for observation of motility.
- Biochemical reactions : Various biochemical reactions like catalase, oxidase, coagulase, carbohydrate fermentation (lactose, glucose, mannitol, sucrose), indole, methyl red, citrate utilization, urease production, H2S production test, etc. were performed as per standard procedures. For all biochemical tests suitable positive and negative control were applied.

Characteristics Of Pathogens Staphylococcus Staphylococcus aureus

It is gram positive cocci (0.5-1.5um) that occurs in irregular grape – like clusters. it is non – motile. The colonies are as follows-

- BA large, smooth, low convex, glistening, densely opaque, of butyrous consistency, golden yellow pigmented, beta hemolytic.
- MA- may or may not ferment lactose

Its identification was confirmed by performing coagluase test.

Streptococcus

Members of genus streptococcus are gram positive cocci arranged in short chains. Most are non-motile, catalase negative and hemolytic.

- BA small, semi-transparent, low convex, discrete with a zone of haemolysis.
- MA- may or may not ferment lactose

S. pyogenes were confirmed by performing catalase, B-hemolysis and further differentiated by bacitracin disk susceptibility.

Enterococcus

Members of genus enterococcus are gram positive cocci arranged in pairs or as short chains. Most are non-motile and non-hemolytic.

- BA pinpoint, circular, translucent
- MA may or may not ferment lactose

Strains or Enterococcus spp were confirmed by performing catalase, growth on 6.5% Nacl, and further differentiated into species.

Klebsiella

Members of the genus Klebsiella, morphologically to be somewhat shorter and thicker than the other enterobacteria. They are straight rods with parallel or bulging sides and rounded or slightly pointed ends. The cells are either in pairs end to end or are arranged singly. They are non-motile, capsulated. They produce abundant gas. The colony characteristics are as follows-

- BA-luxuriant, greyish-white, convex and mucoid colonies.
- MA- lactose fermenting, mucoid colonies.

Proteus

Proteus spp are gram negative bacilli, filamentous, pleomorphic forms though short, fat, coccobacillary forms are not uncommon. They are actively motile. The colony characteristics are as follows :

- BA swarming growth with fishy odour
- MA- non-lactose fermenting colonies.

Citrobacter

Morphologically the strains of citrobacter resemble most other members of the enterobacteriaceae. They are motile organisms. The colony characteristics are as follows:

- BA large, smooth, convex, non-pigmented colonies.
- MA- may or may not ferment lactose.

Pseudomonas Aeruginosa

Pseudomonas aeruginosa is a gram negative, motile, strictly aerobic bacillus. The colonies are as follows:

• BA – large, irregular, feathery, bluish green colonies with earthy smell; diffuse hemolysis may be seen.

- NA- bluish green pigmented, iridescent patches with metallic sheen.
- MA- non-lactose fermenting colonies.
- Antibiotic susceptibility testing:

Antibiotic susceptibility testing of isolated organisms was performed on mueller Hinton agar plates by Kirby Bauer disc diffusion technique as per the CLSI 2013 guidelines.

Mrsa Detection

In the study, MRSA detection was done by Cefoxitin disk diffusion testing.

All the S. aureus isolates were subjects to cefoxitin disk diffusion test using a 30 µg disk. 0.5 McFarland standard suspension of the isolate was prepared and lawn culture done on Mueller-Hinton Agar plates.

Plates were incubated at 37^oC for 18 hour and zone diameters were measured.

Interpretation

Cefoxitin Dis	k Diffusion Test
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	Susceptible	Resistant		
Staph. aureus	\geq 22 mm	\leq 21 mm		

Results and Observations

Out of the hundred cases of pyoderma, primary pyoderma constituted 72% cases and secondary pyoderma 28% cases. The various cases studied include impetigo 13%, folliculits 22%, furuncle 12%, carbuncle 10%, paronychia 6%, cellulitis 9%, acne 5%, infected eczema 7%, infected sebaceous cyst 3%, infected ulcer 5%, infected scabies 8%. The commonest clinical type of pyoderma was folliculitis (22%), followed by impetigo (13%). The incidence was found to be more in males than in females, with the male to female ratio being 2.03:1. Most of the patients belonged to the adolescent age group i.e age group 11-20 years (26%).

Out of 100 samples processed 92% yielded growth whereas 8% did not yield any growth.79 cases (85.8%) showed only one type of growth whereas 13 cases (14.1%) showed two types of organisms. Out of 100 cases the various organisms isolated included S.aureus, CONS, S.pyogenes, Enterococcus, E.coli, klebsiella spp, proteus spp, pseudomonas spp.S.aureus was the commonest organisms isolated accounting for 56% of the total number of cases, it was also the most common isolate from folliculitis (63.63%) and impetigo(69.23%). S.aureus was most sensitive to Vancomycin and Linezolid (100%) followed by Aminoglycosides, it was least sensitive to Penicillin G (14.28%). Resistance to fluoroquinolones as well as to other antibiotics tested was significantly higher in MRSA isolates than that in MSSA isolates (p<0.0001). Among gram negative bacteria, all were uniformly sensitive only to higher antibiotics like Imipenem, Piperacillin, Aztreonam.

Lesions

III. Discussion

In the present study, out of the 100 cases of pyogenic skin infections, primary pyoderma constituted 72% of the cases and the remaining 28% constituted secondary pyoderma. Thus showing that primary pyodermas are more common than secondary pyoderma. Similar finding was reported by other workers^{2,3,7,22}

The reason behind this may be due to timely management of primary skin disorders and traumas. Few studies

have shown almost greater number of secondary than primary pyodermas²³. In present study, folliculitis constituted majority of the cases (22%) followed by impetigo (13%). It is consistent with the work of Patil et al (2006)²⁴ and Paudel et al (2013)² Although a few studies have shown impetigo to be the commonest lesion, which might be because majority of their cases were of pediatric age group.^{22,25}

Incidence of pyoderma in the present study was found to be more in males (67%) than in females (33%). Though there are no explainable reasons for male preponderance in our context, increased outdoor activities of males that subjects them to micro trauma may be a reason for this.

Age

Most of our patients belonged to the adolescent and adult age group. Maximum number of cases fell in the age group 11-20 years (26%), but many studies have found pyodermas to be more common in pediatric age with higher incidence in <10 years age group^{8,9,17} and in few studies > 40% patients belong to 1-4 years age

group^{5,7}. As most of the pediatric patients specifically visit the pediatric and surgical out patient department for minor skin problems, this may be the reason for a low number of pyodermas in children in this study

Obtained

Out of 100 samples processed in the present study 92 cases (92%) yielded growth whereas 8 cases (8%) did not yield any growth. Similar findings were reported by Paudel et al² 93.3% growth rate while Gandhi et al²⁵ observed culture positivity in 91.5% cases. Out of the 92 culture positive cases, a single infecting organism was isolated from 79 cases (86%) and mixed isolate were obtained from the remaining 13 cases (14%). Similar findings were noted by other workers.^{5,7,9} a few workers, however have isolated a higher percentage of mixed organism than single organism.³

Aetiological Agents

In the present study conducted on 100 cases the most common pathogen isolated was S.aureus (56%). Similar findings have been reported by other workers.^{3,5,9,14,17,24} However, there was no significant difference between the isolation of S.aureus in primary and secondary pyoderma, the percentage being 62.5% & 39.3% respectively with a P>0.005 which correlates with the study of Paudel et al.² in one study, even in chronic wound infections, S.aureus was isolated in 70.8% of cases, though more number of gram negative bacilli have been isolated from secondary pyodermas and chronic wound infections as compared to primary pyodermas.²

Isolation of Streptococci in present study was 11% which is similar to that of Chopara et al.²⁴ where the isolation was 13.3%. However other studies^{3,5,9,14,24} have shown a higher as well as lower isolation rate. The reason behind this could be due to the change in the etiological agents or due to inhibition of S.pyogenes by secondary invasion of S.aureus which is supposed to produce bacteriocins, toxic to Streptococci or due to bacterial interference.

In our study Enterococcus spp were isolated in 4% cases which is similar to study conducted by Malhotra et al.²³ In study conducted by Ramana et al¹⁴ isolation rate of Enterococcus spp was 11.4%. as Enterococcus fecalis is a part of normal fecal flora, the isolation seen in this study may be due to contamination of the lesion or due to opportunistic infection, 2 in our study, 10% were CONS, 2% E.coli, 10% Klebsiella spp, 1% Proteus spp and 6% Pseudomonas spp were found.

In our study,out of the 22 cases of folliculitis, S.aureus was isolated from 14 cases (63.03%), S. Pyogenes 2(9%), klebsiella spp was isolated from 4 (18%) cases. This is comparable to Gandhi et al²⁵ who isolated 71.1% caused by S.aureus (56) followed by β - haemolytic Streptococci (1.2%), 8.9% samples grew klebsiella spp.

Similarly, 69.23% impetigo cases grew S.aureus alone, while Gandhi et al²⁵ isolated S.aureus from 81.1% impetigo cases, Patil et al²⁴ from 58.9 cases.

Antibiotic Susceptibility Pattern

S.aureus was 100% sensitive to Linezolid and Vancomycin followed by Netilmicin (96.4%), Amikacin(99.6%), Tobramycin(85.7%), Gentamicin (85.7%). Aminoglycosides were followed by Clindamycin, Tetracyclin and erythromycin in range of 80-90% sensitivities. While Ciprofloxacin(55.35%), Chloramphenicol (37.5%) and Cotrimoxazole(35.7%) were found to be less sensitive drugs. This is compared to other studies . Penicillin is least sensitive ^(14.26) probably is due to the penicillinase

This is compared to other studies . Penicillin is least sensitive ^(14.26) probably is due to the penicillinase producing strains. Similar findings have been shown by other workers.^{3,5}

Among the CONS isolated, 100% were sensitive to Linezolid and Vancomycin whereas Amioglycosides, Quinolones, Cotrimoxazole and Cephalosporins show sensitivity in range 80%-90%. It was least sensitive to Penicillin G (10%). Malhotra et al $(2012)^{27}$ also suggested high (77.7%) aminoglycoside sensitivity.

All S.pyogenes isolates were sensitive most of the antibiotics tested. Malhotra et al (2012)²⁷ observed similar findings.

Enterococci were most resistance to Penicillin G and Ampicillin (25%). Paudel et al² (2013) also showed 100% resistance to this drugs.

Among gram negative bacterias, all were uniformly sensitive only to higher antibiotics like Imipenem, Piperacillin, Aztreonam. Least effective drugs were Ampicillin and Amoxyclav. Proteus.spp, showed increased resistance to all antibiotics with 100% resistance to Cefoxitin, Cefotaxim, Tetracyclin,Chloramphenicol and Cotrimoxazole.

Percentage Of MRSA Isolated

In present study, percentage of MRSA isolated was 12.5%. Thus, showing that the incidence of MRSA from pyoderma cases was not very high. Similar findings have been shown by several workers who have also shown a decreased incidence of MRSA^{14,24,28}

Resistance Pattern Of MRSA And MSSA

Comparision of antibiotic resistance pattern among MRSA and methicillin sensitive S.aureus (MSSA) isolates showed that resistance to fluroquinolones as well as to other antibiotics tested as significantly higher in MRSA isolates than that in MSSA isolates (p<0.0001) Table . similar findings were observed by Nishijima et al $(2002)^{29}$ and Subedi et al (2005).³⁰

The MSSA isolates were susceptible to most of the antibiotics tested, although high degree of resistance was observed with penicillin (83.6%), cotrimoxazole (53.06%) and Ciprofloxacin (30.6%) the antibiotics often used to treat general infections. The present study shows a relatively high rate of susceptibility pattern among the clinically isolated MSSA to tetracycline, aminoglycosides and macrolides.

IV. Conclusion

Pyogenic skin infections are frequently encountered in day to day clinical practice. Multi drug resistance 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. Multi drug resistance has resulted from indiscriminate use of antibiotics. A correct 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 resistance 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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Staphylococcus aureus	CONS	E	S.p.	E.Coli	К	Р	Ps.	S.a + S.p	S.a + K	K + Ps	N.G.
7 (53.8%)	2 (15.38%)	-	-	-	-	-	-	1 (7.69%)	1 (7.69%)	-	2 (15.38)
11 (50%)	1 (4.54%)				3 (13.63%)	1 (4.54%)		2 (9.09%)	1 (4.54%)	-	3(13.63%)
8 (66.66%)	3 (25%)	-	1 (8.33%)	-	-	-	-	-	-	-	-
7 (70%)	-	1 (10%)	2(20%)	-	-	-	-	-	-	-	-
4 (66.6%)	-			2 (33.3%)	-	-	-	-	-	-	-
1 (11.11%)	-	3 (33.3%)	2 (22.22%)	-	1 (11.11%)	-	-	1 (11.11%)	1 (11.11%)	-	-
3 (60%)	1 (20%)										1 (20%)
-	-	-	1 (14.28%)	-	-	-	3 (42.85%)	1 (14.28%)	-	-	2 (28.57%)
2 (66.6%)	-	-	-	-	-	-	-	-	1 (33.3)	-	-
-	-	-	-	-	-	-	1 (20%)	-	2 (40%)	2(40%)	-
5 (62.5%)	3 (37.5%)	-	-	-	-	-	-	-	-	-	-

Bacterial Isolates From Various Pyodermas

Antimicrobial Sensitivity Of Gram Positive Cocci From Pyodermas

Drugs	S. aureus n=56 (%)	CONS n=10 (%)	S. pyogenes n =11 (%)	Enterococcus
Penicillin G	8 (14.28%)	1 (10%)	6 (54.5%)	1 (25%)
Ampicillin				1 (25%)
Cefoxitin	49 (87.5%)	8 (80%)	-	
Cefepime			9 (81.8%)	
Erythromycin	40 (83%)	7 (70%)	7(63.6%)	
Clindamycin	47 (83.92%)	8 (80%)	8 (72.7%)	
Gentamicin	48 (85.7%)	8 (80%)		1 (25%)
Amikacin	53 (94.6%)	9 (90%)		
Tobramycin	48 (85.7%)	8 (80%)		
Netilmicin	54 (96.4%)	8 (80%)		
Tetracyclin	47 (83.92%)	7 (70%)		
Chloramphenicol	21 (37.5%)	4 (40%)	7 (63.6%)	
Ciprofloxacin	31 (55.35%)	9 (90%)		
Cotrimoxazole	20 (35.7%)	8 (80%)		
Vancomycin	56 (100%)	10 (100%)	11 (100%)	4 (100%)
Linezolid	56 (100%)	10 (100%)	11 (100%)	4 (100%)

	-	_		
Drugs	E.coli n=2 (%)	Klebsiella spp n=10 (%)	Proteus Spp n =11 (%)	Pseudmonas Spp n=6 (%)
Ampicillin	0 (0%)	0(0%)	1 (100%)	0 (0%)
Amoxyclav	0 (0%)	1 (10%)	1 (100%)	1 (16.6%)
Cefuroxime	0 (0%)	2 (20%)	1 (100%)	-
Cefoxitin	1 (50%)	7 (70%)	0 (0%)	3 (50%)
Cefotaxim	1 (50%)	8 (80%)	0 (0%)	-
Cefepime	1 (50%)	7 (70%)	1 (100%)	4 (66.6%)
Ceftazidime	1 (50%)	5 (50%)	1 (100%)	3 (50%)
Pipercillin	2 (100%)	8 (80%)	1 (100%)	3 (50%)
Imipenem	2 (100%)	10 (100%)	1 (100%)	5 (83.3%)
Azteronam	2 (100%)	9 (90%)	1 (100%)	5 (83.3%)
Gentamicin	2 (100%)	3 (30%)	1 (100%)	3 (50%)
Amikacin	2 (100%)	5 (50%)	0 (0%)	3 (50%)
Tobramycin	1 (50%)	4 (40%)	0 (0%)	2 (33.3%)
Netilmicin	2 (100%)	9 (90%)	1 (100%)	2 (33.3%)
Tetracyllin	1 (50%)	1 (10%)	0 (0%)	-
Chloramphenicol	1 (50%)	5 (50%)	0 (0%)	-
Ciprofloxacin	1 (50%)	5 (50%)	1 (100%)	4 (66.6%)
Cotrimoxazole	0 (0%)	5 (50%)	0 (0%)	1 (16.6%)

Antimicrobial Sensitivity Of Gram Negative Bacteria From Pyoderma