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Research Article

Bacteriological profile of pyoderma in a tertiary care centre in Kerala, India

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ABSTRACT

Background: Pyoderma is a leading cause of childhood infection, which also affects the adults with a changing trend of antibiotic sensitivity pattern of the bacterial agent. A study on the bacteriological profile of pyoderma and its antibiotic sensitivity pattern is beneficial as it is the first study of its kind in this study centre. The main objective of the study was to find the bacteriological profile and the antibiotic sensitivity pattern of the isolates.
Methods: A descriptive study was conducted during a one year period at Government medical college, Kottayam, India. 150 cases were studied. Data was collected to assess the risk factors. Pus collected from the site of infection was cultured, the organisms were identified and their antibiotic sensitivity pattern was found by conventional methods.
Results: There were 173 isolates obtained from 150 patients. The predominant isolate obtained was *Staphylococcus aureus* including 13% of MRSA, followed by *Beta hemolytic Streptococci*. Other isolates obtained were *Pseudomonas aeruginosa* and *Citrobacter amalonaticus*.
Conclusion: The most common organism causing pyoderma is found to be *Staphylococcus aureus* which include 13.3% of MRSA. The next common isolate obtained is *Beta hemolytic Streptococci.* 38 cases showed mixed infections including few gram negative bacilli. So this study emphasizes the need for pus culture and sensitivity which facilitates the appropriate usage of antimicrobial agents which can prevent emergence of antimicrobial resistance.

Key words: Pyoderma, Antimicrobial resistance, *Staphylococcus aureus*

INTRODUCTION

Pyoderma is a cutaneous bacterial infection associated with elevated polymorph nuclear count. Pyodermas are one of the commonest clinical conditions encountered in dermatological practices especially in pediatric age group.

Various factors like poverty, malnutrition, overcrowding and poor hygiene have been stated to be responsible for its high incidence in the lower socioeconomic strata. Climatic conditions also play a role with hot and rainy seasons being period of maximum occurrence. Pyoderma is classified into primary and secondary. Primary pyoderma is a pyogenic infection of the normal skin and its appendages. It constitutes impetigo contagiosa, bullous impetigo, ecthyma, superficial folliculitis, furunculosis, and carbuncle. Secondary pyodermas include infected scabies, infectious excematoid dermatitis etc.

Pyoderma is usually caused by coagulase positive *Staphylococci* gram *beta-haemolytic streptococci* and less commonly by gram negative organisms. For successful treatment of cases of pyoderma a detailed knowledge about the causative microorganisms is needed.

Most cases of pyoderma do not respond to the antibiotics that were previously very effective for such cases. Indiscriminate use of topical and systemic antibiotics and inadequate use of systemic antibiotics have contributed to this situation. Increasing resistance to antibiotics seen in microorganisms poses a big problem to the clinicians.

Keeping this view in mind the present study is designed.

METHODS

It was a descriptive study conducted at Department of Microbiology Government Medical College, Kottayam, India and Department of dermato venereo leprology Government medical College, Kottayam, India, for one year from February 2012.

Sample size was calculated using epi-info software with expected frequency 10% and absolute precision 5. The sample size obtained was 138 with 95% confidence interval. This figure was rounded and total 150 samples were collected.

Inclusion criteria

All the cases of pyoderma attending Department of Dermatology during a period of one year from February 2012.

Exclusion criteria

Debilating illness like renal failure or AIDS.

Procedure

For sample collection a total of 150 cases were studied. The patients included were all the patients attending Dermatology department with primary and secondary pyoderma; during the study period. The details of the patients were entered in the proforma and consent was taken before collecting the sample.

The site of infection was cleaned thoroughly with sterile normal saline. The deeply expressed pus was then collected using sterile double swab and transported to the laboratory immediately.

Processing of samples

On reaching the laboratory the samples were immediately processed. Gram staining was done using one swab. Other swab was used for aerobic culture.

Culture was done on 5% sheep blood agar, chocolate agar, MacConkey agar, salt agar and glucose broth.

The blood agar and chocolate agar plates were incubated at 37^{o}C under 5% CO_2 using CO_2 generating sachet (GENbag CO_2 bioMerieux); MacConkey agar and salt agar and glucose broth were incubated at 37^{o}C. Growth was examined after overnight incubation and at 48 hours.

Identification of the bacterial pathogen (aerobes and facultative anaerobes)

The aerobic cultures were examined after 18-24 hours and 48 hours. The identification of the bacterial pathogens were done based on staining, cultural and biochemical properties using standard laboratory procedures.[1]

Lancefield grouping of Streptococci

The test was done using SLIDEX Strepto Plus Kit (Biomerieux). It is a latex agglutination test for the grouping of Lancefield group A, B, C, D, F and G Streptococci. Isolated colonies (3-4 colonies) of Streptococci are placed in a tube containing 400µl of extraction enzyme and incubated at 37^{0}C for 10 minutes .The group specific antigen is enzymatically extracted from the streptococcal cell wall. Antigen in the extract is identified using latex particles sensitized with group specific anti-streptococcal antibody.

The procedure followed is slide agglutination in which 15 microlitres of extracted antigen is added to a drop of grouping antigen provided and is thoroughly mixed. It is then rocked gently for 2 minutes and look for the presence of aggregates. Visible aggregates will be formed in the specific latex particle suspension which reacts with the extracted antigen. The latex will remain in the suspension if the antigen is not present in the extract.

Antibiotic sensitivity testing

Antibiotic sensitivity testing of bacterial isolates was done using Stokes and Kirby-Bauer method. The antibiotics selected were according to organism isolated. Mueller Hinton Agar was used for sensitivity testing except for *Streptococcus* spp. for which 5% sheep blood agar was used. Antibiotic sensitivity testing of the isolate was done by standard disc diffusion method. The inoculums were standardized by comparing with 0.5 McFarland's opacity standard. Control organisms were respective ATCC strains.

Plates were incubated at 37^{o}C overnight and results were read by comparing the zone of inhibition of control and test strains and reported accordingly.

Detection of methicillin resistant Staphylococcus aureus

The methicillin resistance in *Staphylococcus aureus* isolates were tested according to CLSI guidelines.

Disc diffusion test for the antibiotics Cefoxitin -30 µg was performed. The zone diameter of less than 22 mm was taken as resistant and a zone of more than 23mm was taken as sensitive.

For those isolates found to be resistant by disc diffusion test using cefoxitin disc was further confirmed by performing epsilometer (E-Test) with cefoxitin and oxacillin drugs and their MICs were detected

Test for sensitivity to vancomycin

Vancomycin sensitivity of *Staphylococcus aureus* was tested by doing agar dilution and epsilometer test.

Agar dilution test for vancomycin

Antimicrobial dilutions were prepared and poured into unsupplemented Mueller Hinton agar as per CLSI guidelines The suspensions of test and control organisms with 0.5 Macfarland turbidity were prepared and 1 microlitre of each suspension were inoculated onto each agar plate.The plates were incubated overnight at 37^0c . The concentration of antibiotic at which no growth is seen is taken as the MIC of vancomycin for the organism tested (Figure 29).

Test for inducible clindamycin resistance

Inducible clindamycin resistance of *Staphylococcus aureus* was detected by doing lawn culture of the organism in Mueller Hinton agar plate and placing the discs of erythromycin (15µg) and clindamycin (2µg) at a distance of 26 mm. The plates were incubated at 37^0C overnight. Flattening of zone of inhibition of clindamycin adjacent to erythromycin disc (to as D-zone) was noted in isolates with inducible clindamycin resistance.[2]

RESULTS

As per the inclusion and exclusion criteria, pus was collected from 150 patients who had pyoderma.

Table 1: Total isolates obtained in pyoderma.

Type	No	Percentage
Monomicrobial	97	64.66%
Polymicrobial	38	25.33%
Sterile	15	10%
Total	150	100%

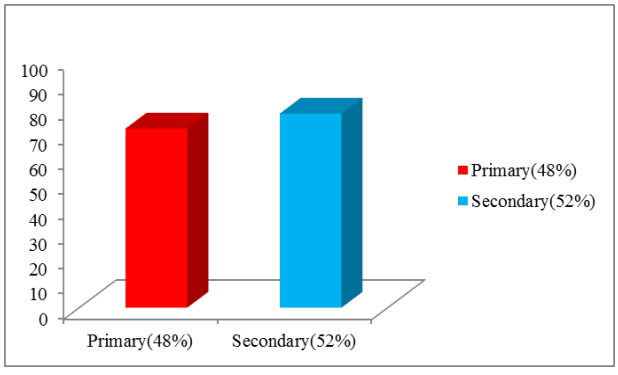

Figure 1: Types of pyoderma.

Table 2: Age distribution in pyoderma.

Age group in years	Number	Percentage
0-15	50	33.33%
16-30	33	22%
31-45	21	14%
46-60	36	24%
61-75	9	6%
76-90	1	0.66%
Total	150	100%

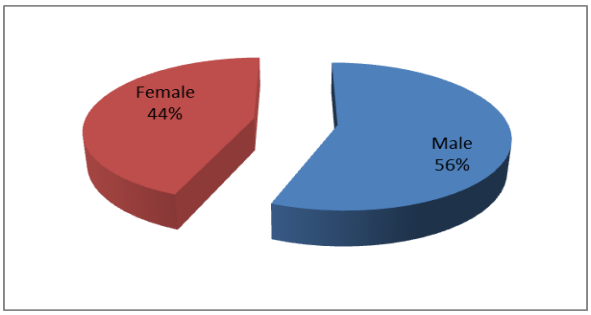

Figure 2: Gender distribution in pyoderma.

Table 3: Type of secondary pyoderma

Type	Number	Percentage
Venous ulcer	25	32.05%
Scabies -2^0 infection	7	8.9%
Eczema	38	48.71%
Trophic ulcer	1	1.2%
Insect bite reaction	5	6.4%
Pemphigus 2^0 infection	2	2.5%
Total	78	100%

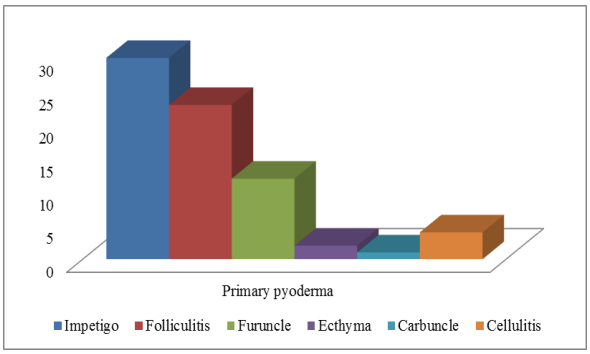

Figure 3: Types of primary pyoderma.

Table 4: Sites affected in pyoderma.

Site	Number	Percentage
Scalp	7	4.66%
Face	14	9.33%
Trunk	9	6%
Upper limb	25	16.66%
Lower limb	95	63.33%
Total	150	100%

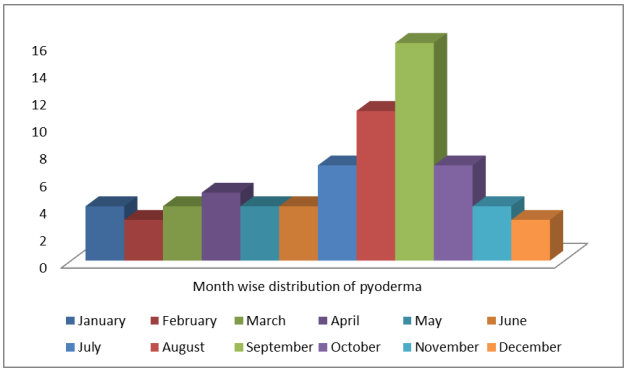

Figure 4: Month wise distribution of pyoderma.

Table 5: Risk factors involved in pyoderma.

Risk factors	Number	Percentage
Atopy	10	6.66%
Diabetes	26	17.33%
Venous insufficiency	8	5.33%
Trauma	6	4%
Immunosupression	8	5.33%
Nil	92	61.33%
Total	150	100%

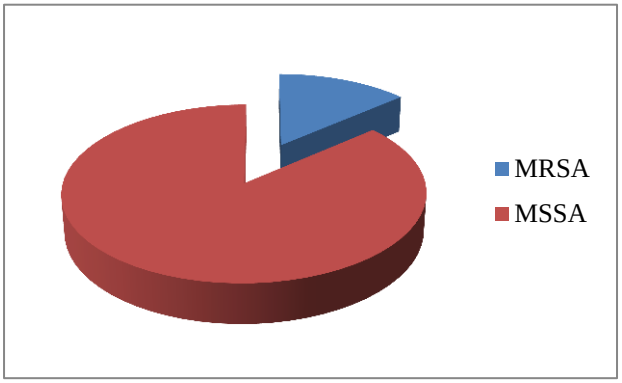

Figure 5: Percentage of MRSA isolates.

Table 6: Microbiology of Pyoderma.

Organism	Number	Percentage
Staphylococcus aureus	117	67.63%
Group A Beta haemolytic Streptococci	50	28.9%
Group G Beta haemolytic Streptococci	1	0.57%
Staphylococcus epidermidis	1	0.57%
Staphylococcus hemolyticus	1	0.57%
Citrobacter amalonaticus	1	0.57%
Pseudomonas aeruginosa	2	1.15%
Total	173	100%

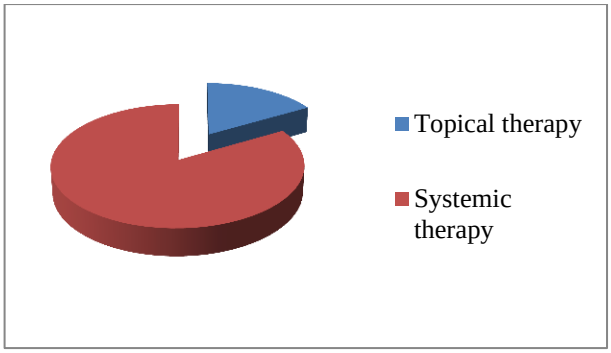

Figure 6: Treatment given.

Table 7: Microbiology of primary pyoderma.

Organism	Number	Percentage
Staphylococcus aureus	52	74.28%
Group A Beta haemolytic Streptococci	14	20%
Staphylococcuc epidermidis	1	1.4%
Staphylococcus hemolyticus	1	1.4%
Citrobacter amalonaticus	1	1.4%
Pseudomonas aeruginosa	1	1.4%
Total	72	100%

Table 8: Microbiology of secondary pyoderma.

Organism	Number	Percentage
Staphylococcus aureus	65	63.1%
Group A Beta haemolytic Streptococci	36	34.95%
Group G Beta haemolytic Streptococci	1	0.97%
Pseudomonas aeruginosa	1	0.97%
Total	78	100%

Table 9: Lancefield groups of *Beta haemolytic Streptococci* obtained.

Group	Number	Percentage
A	50	98.03%
G	1	I.94%
Total	51	100%

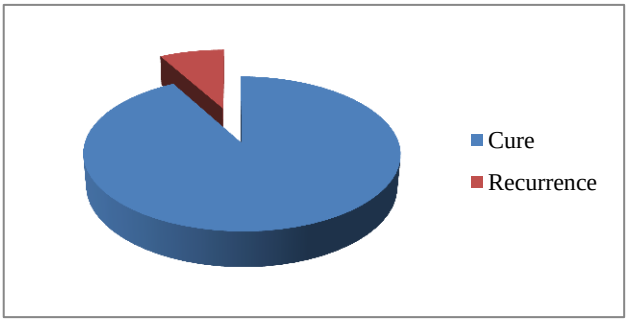

Figure 7: Outcome of Treatment.

Table 10: Antibiotic sensitivity pattern of *Staphylococcus* species.

Antibioitics	MRSA	MSSA	*Staphylococcus epidermidis*	*Staphylococcus hemolyticus*
Penicillin(10 u)	0	0	0	0
Gentamicin	2 (12.5%)	67 (66.33%)	1 (100%)	1 (100%)
Amikacin	9 (56.25%)	100 (99%)	1 (100%)	1 (100%)
Erythromycin	0 (0%)	45 (44.55%)	1 (100%)	1 (100%)
Cefoxitin	0	101 (100%)	1 (100%)	1 (100%)
Vancomycin	16 (100%)	101 (100%)	1 (100%)	1 (100%)
Tetracycline	16 (100%)	101 (100%)	1 (100%)	1 (100%)
Linezolid	16 (100%)	101 (100%)	1 (100%)	1 (100%)
Clindamycin	16 (100%)	101 (100%)	1 (100%)	1 (100%)
Rifampicin	16 (100%)	101 (100%)	1 (100%)	1 (100%)
Teicoplanin	16 (100%)	101 (100%)	1 (100%)	1 (100%)

Table 11: Antibiotic sensitivity pattern of *Beta haemolytic Streptococci.*

Antibiotic	*Group A Streptococci*	*Group G Streptococci*
Pencillin	50 (100%)	1 (100%)
Erythromycin	50 (100%)	1 (100%)
Vancomycin	50 (100%)	1 (100%)
Clindamycin	50 (100%)	1 (100%)
Linezolid	50 (100%)	1 (100%)
Tetracycline	50 (100%)	1 (100%)

Table 12: Antibiotic sensitivity of gram negative bacilli obtained.

Antibiotic	*Citrobacter*	*Pseudomonas*
Ampicillin	0	NT (not tested)
Gentamicin	1 (100%)	0
Amikacin	1 (100%)	1 (50%)
Cephalosporin-1st generation	0	NT
Ceftazidime	NT	2 (100%)
Cefotaxime	1 (100%)	NT
Ciprofloxacin	1 (100%)	1 (100%)
Cefoperazone-Sulbactum	1 (100%)	NT
Piperacillin-Tazobactum	1 (100%)	1 (100%)
Meropenem	1 (100%)	1 (100%)

Types of primary pyoderma

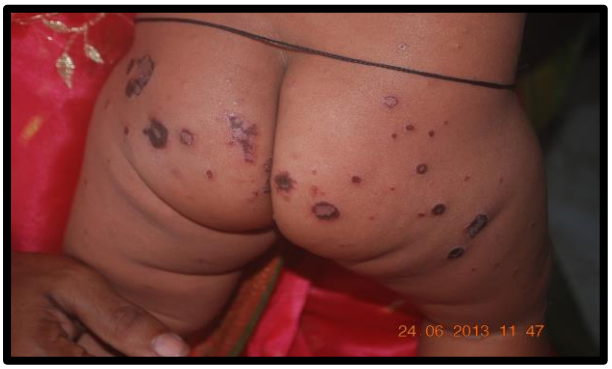

Figure 8: Impetigo.

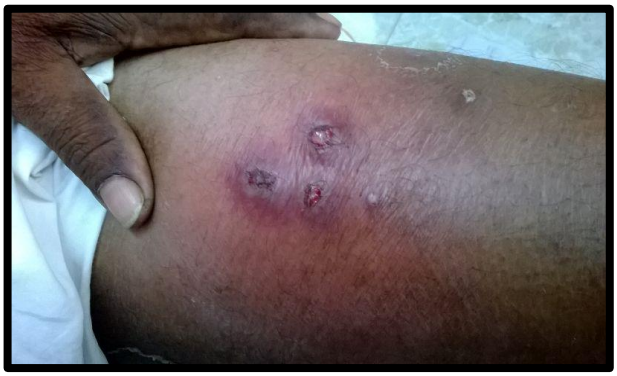

Figure 9: Furuncle.

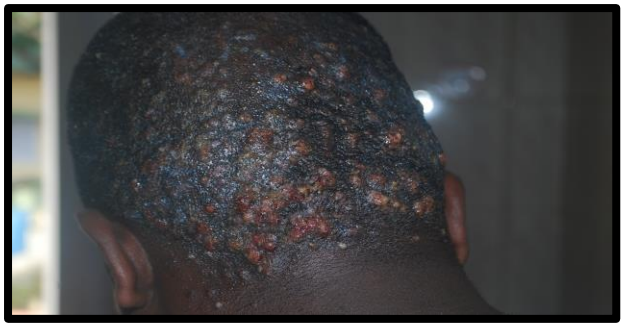

Figure 10: Folliculitis.

Types of secondary pyoderma

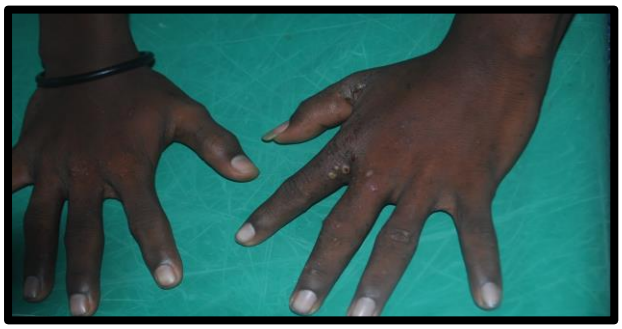

Figure 11: Scabies secondary infection.

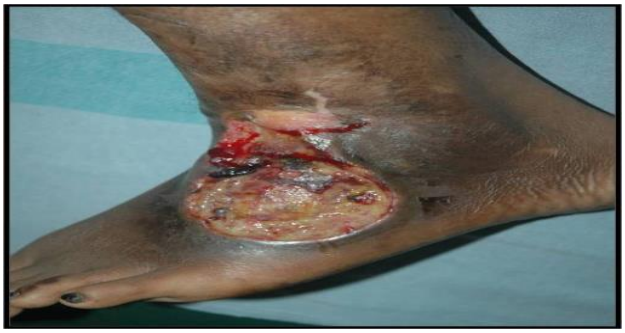

Figure 12: Venous ulcer.

Identification of Beta hemolytic Streptococci

Figure 13: GENbag CO_2 (Bio-Meriux) with 5% sheep blood.

Figure: 14 SLIDEX Strepto Plus kit (Bio-Meriux).

Figure 15: Beta hemolytic colonies of *Streptococcus pyogenes.*

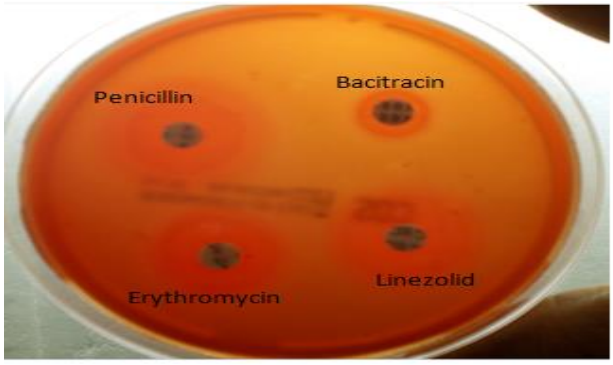

Figure 16: Sensitivity pattern of *Streptococcus pyogenes.*

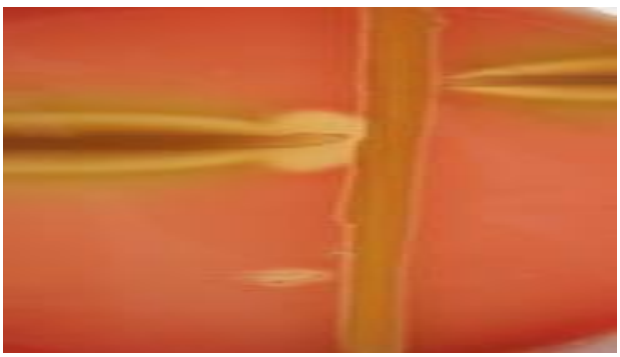

Figure 17: CAMP test.

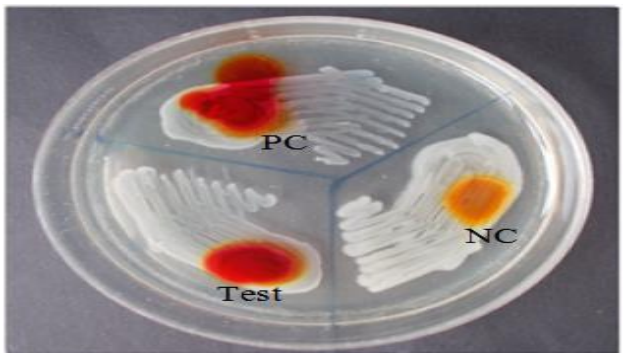

Figure 18: PYR test.

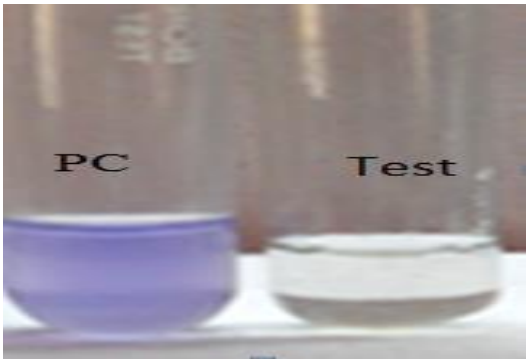

Figure 19: Hippurate hydrolysis.

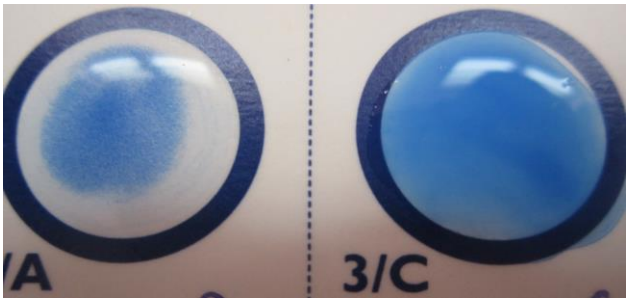

Figure 20: Latex agglutination test for grouping of *Streptococci.*

Identification of Staphylococcus aureus

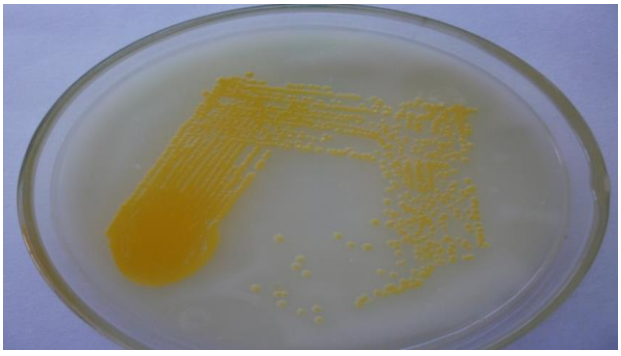

Figure 21: Colonies of *Staphylococcus aureus* on salt milk agar.

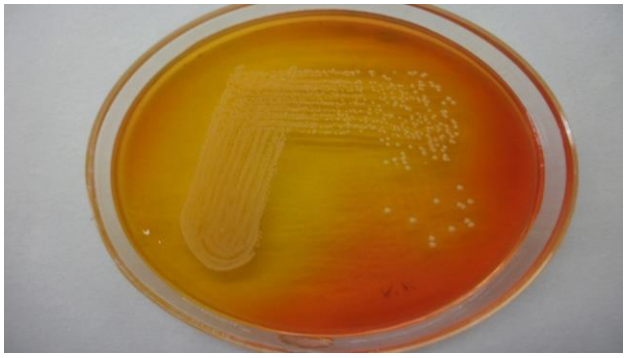

Figure 22: Colonies of *Staphylococcus aureus* on mannitol salt agar.

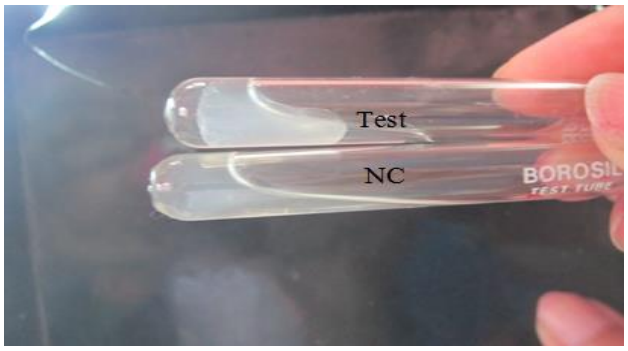

Figure 23: Coagulase test.

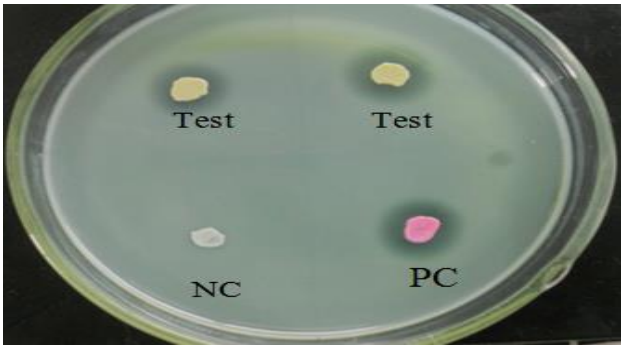

Figure 24: DNAase test.

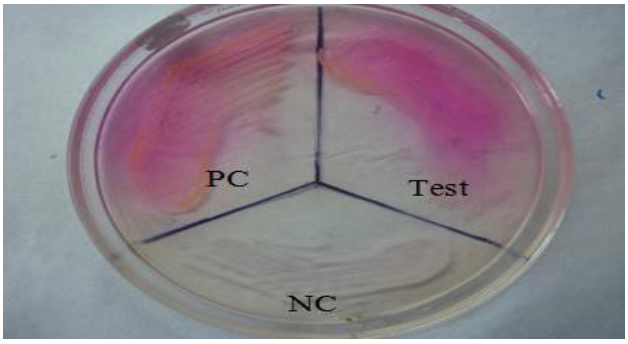

Figure 25: Phosphatase test.

Antibilotic susceptibility testing of Staphylococcus aureus

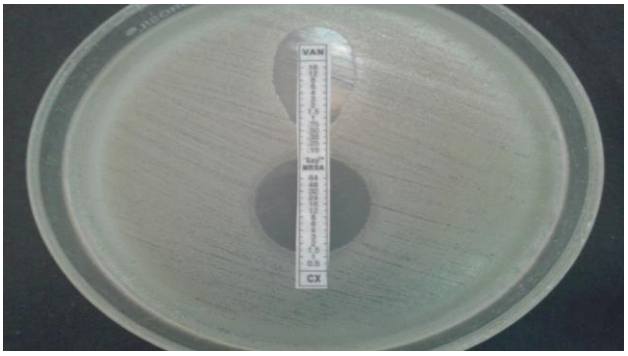

Figure 26: Epsilometer test of MSSA

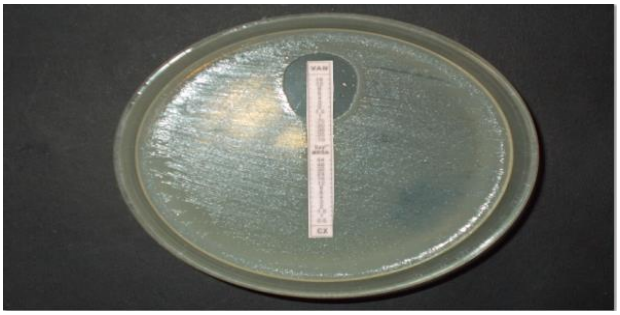

Figure 27: Epsilometer test of MRSA.

Figure 28: D test for inducible clindamycin resistance.

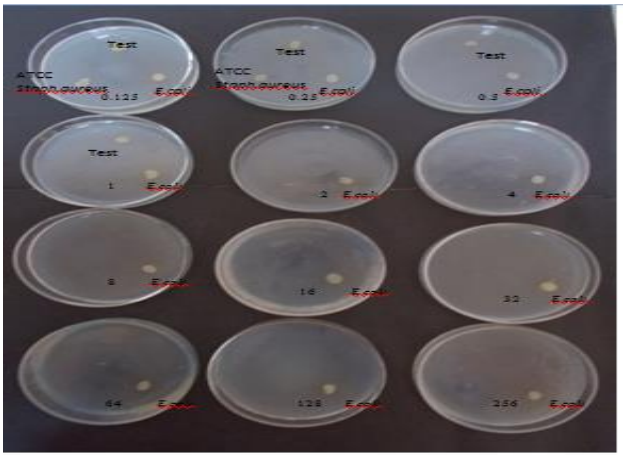

Figure 29: Vancomycin agar dilution test to detect MIC

DISCUSSION

A one year study was conducted to analyse the bacteriological profile of pyoderma in Government Medical College, Kottayam, India. A total of 150 cases of Pyoderma were studied and 173 isolates were obtained.

In the present study of 150 cases, 72 cases were primary pyoderma and 78 cases were secondary pyoderma. In this study both primary and secondary pyodermas were most common among the age group of 0-15 years. This finding correlated with study conducted by Parikh DA et al in.[3] It was least common among elder age group of 76-90 years.

Pyoderma was more common among males (56%) than females in the present study. In several other studies conducted by Ghadage DP et al in and Andrews RM et al in also similar pattern of gender distribution was obtained.[4,5]

In the study, among a total of 150 cases, 135 yielded growth while 15 were sterile. Among these 15 cases 13 were already started on antibiotics and 2 cases were secondary infection following insect bite reaction which might be an allergic response.

Of the 135 cases which yielded growth, 97 cases showed monomicrobial infection while 38 cases showed polymicrobial infection. Among the monomicrobial infection primary pyodermas were more common 52 (53.6%) than secondary whereas among the polymicrobial infection secondary pyodermas were more common than primary.

Among a total of 72 cases of primary pyodermas which were included in the study, most common case were impetigo (41.66%). This finding was also obtained in various other studies including the study conducted by Mathew SM, Garg B et al at Pondichery in the year.[6]

Other cases of primary pyodermas studied include folliculitis (31.9%), furuncle (16.66%), cellulitis (5.55%) ecthyma (2.77%), and carbuncle (1.38%) in the decreasing order of frequency obtained in this study.

Among a total of 78 cases of secondary pyodermas studied most common case were eczema (48.1%). This finding was similar to the study conducted by Parikh DA, Fernandez RJ at Bombay.[3] Other cases of secondary pyodermas include venous ulcer (32.05%), scabies-secondary infection (8.97%), Insect bite reaction (6.41%), pemphigus-secondary infection (2.56%) and trophic ulcer (1.2%) in the decreasing order of frequency obtained.

In the present study the most common site affected were lower limbs (63.33%). This finding is similar to many other studies on pyoderma including study conducted by Gandhi S et al.[7]

The least common site affected was scalp (4.66%). Other areas affected include upper limb (16.66%), face (9.33%) and trunk (6%).

Considering the presence of risk factors which predisposed to the occurrence of pyoderma in the present study, 58 cases had some risk factor for pyoderma whereas there was no risk factor in 92 cases obtained. Most common risk factor in this study was diabetes (17.33%). Other risk factors were atopy (6.66%), venous insufficiency (5.33%), trauma (4%) and immunosuppression (5.33%). No risk factor for pyoderma was present in 92 (61.33%) cases obtained.

Considering the microbiological profile in the study, a total of 173 isolates were obtained. 70 isolates were obtained from a total of 72 primary pyoderma cases and 103 isolates were obtained from a total of 78 secondary pyoderma cases studied. Polymicrobial infections were more in secondary pyoderma compared to primary pyoderma.

Most common isolate obtained was *Staphylococcus aureus* (67.63%). Among this 79 (52.66%) isolates were obtained as single organism i.e. monomicrobial infection were as 38 (25.33%) isolates were obtained in polymicrobial infection.

Next common isolate was *Beta haemolytic Streptococcci* (29.47%). This was similar to the study conducted in Pondichery by Khalil A.[8] Among the *Beta haemolytic Streptococci*, group A constituted the commonest isolate (98.03%). Only other group of *Beta haemolytic Streotococci* obtained was group G from a case of trophic ulcer.

Other isolates obtained include Coagulase negative *Staphylococci* which included *Staphylococcus hemolyticus* (0.57%) and *Staphylococcus epidermidis* (0.57%), *Pseudomonas aeruginosa* (1.15%), *Citrobacter amalonaticus* (0.57%).

Considering pyodermas the primary pyoderma, most common isolate was *Staphylococcus aureus* (74.2%). The similar result is also obtained in a study conducted on primary pyodermas by Ali MK at Iraq.[9] The next common isolate obtained was *Beta haemolytic Streptococci* (20%).

In secondary pyodermas also most common isolate obtained was *Staphylococcus aureus* (63%) followed by *Beta haemolytic Streptococci* (34.95%).[10] A total of 117 isolates of *Staphylococcus aureus* were obtained among which 16 isolates were methicillin resistant strains and 101 isolates were methicillin sensitive. Only community acquired cases coming as outpatients from the community were included in the study. So according to this study the number of community acquired MRSA causing pyodermas are increasing .This correlates with various other studies on MRSA.[11-13]

Only one isolate of the family *Enterobacteriacea* was obtained which is *Citrobacter amalonaticus*. Two isolates of *Pseudomonas aeruginosa* was obtained. One case was that of Ecthyma. *Pseudomonas aeruginosa* was isolated from cases of ecthyma in other studies including the study conducted by Singh TN et al.[14] The other case was a secondary infection in the lower limb in a diabetic patient who is a farmer by occupation.

Antibiotic susceptibility

Antibiotic sensitivity pattern of the isolates were studied in detail. There is an emerging trend of increase in the antibiotic resistance in almost all organisms as suggested by the literature reviews. In the present study also most of the isolates obtained were resistant to multiple antibiotics.

Staphylococcus aureus

Among the total 117 isolates of *Staphylococcus aureus*, 101 isolates were sensitive to cefoxitin i.e. *methicillin sensitive Staphylococcus aureus* (MSSA) and 16 (13%) isolates were resistant to cefoxitin i.e. *methicillin resistant Staphylococcus aureus*. This finding correlates with the study conducted on Community acquired MRSA by Jenkins TC et al and Ho PL et al at Hong Kong.[15,16]

Among the MSSA 67 (66.33%) were sensitive to Gentamicin. Similar result was obtained in a study conducted by Malhotra SK et al, at Amritsar, Punjab and another study conducted by Gupta V et al at.[17,18] 101 (99%) were sensitive to Amikacin. Only 45 (44.55%) cases of MSSA were sensitive to Erythromycin.

There were 100% sensitivity to vancomycin, linezolid, tetracycline, clindamycin, rifampicin and teicoplanin. This finding is similar to that obtained by Daun RS et al in a study conducted at U.S and Canada.[19] None of them was sensitive to Penicillin. Among the total 16 MRSA isolates obtained only 2 (12.5%) were sensitive to Gentamicin, whereas 9 (56.25%) were sensitive to Amikacin. None of them were sensitive to Erythromycin.

There were 100% sensitivity to vancomycin, linezolid, clindamycin, tetracycline, rifampicin and teicoplanin for the MRSA isolates. This finding correlates with studies conducted on MRSA which was included in the study by Ellington MJ et al, and Moran GJ et al.[20,21]

Among 55% of erythromycin resistant strains 12% showed inducible clindamycin resistance by D test. This finding correlates with the study conducted by Gupta V et al at Chandigarh.[19,22]

Beta haemolytic Streptococci

Among a total of 51 isolates of *Beta haemolytic Streptococci*, 50 were Group A (*Streptococcus pyogenes*) and one isolate was Group G. Group G Streptococci has been isolated from cases of pyoderma in other studies

also.[23] All of the beta haemolytic Streptococci showed 100% sensitivity to all antibiotics tested, which include penicillin, erythromycin, cephalosporins, vancomycin, linezolid, tetracycline, teicoplanin.[23]

Pseudomonas aeruginosa

Among the 2 isolates of *Pseudomonas aeruginosa* obtained both were resistant to Gentamicin. One isolate (50%) was sensitive to Amikacin. Both of them were sensitive to other antibiotics tested which include Ceftazidime, Ciprofloxacin, Piperacillin-Tazobactum, Meropenem.

Enterobacteriaceae

Only one isolate from enterobacteriaceae family was obtained which is *Citrobacter amalonaticus*. The isolate was sensitive to Gentamicin, Amikacin, Third generation Cephalosporins, Ciprofloxacin, Piperacillin-Tazobactum, Cefoperazone-Sulbactum and Meropenem. It was resistant to Ampicillin.

Outcome

Out of 150 cases, 25 cases were given topical treatment based on the clinical presentation. 125 cases were started on empirical therapy after collecting sample for culture and the patients were asked to come for review on third day.

The empirical treatment given for almost all cases were Ampicillin - Cloxacillin combination. On the third day the MRSA infections showed no signs of clinical cure and the antibiotic was changed to Linezolid given for duration of 2 weeks.

Among the 16 cases of MRSA infection 12 showed a recurrence of infection. Recurrence of cutaneous MRSA infections is also noted in other studies on MRSA.[24] The empirical treatment with ampiclox was changed, in the second visit on the third day for the cases from which Gram negative bacilli was isolated. Treatment was given according to the sensitivity report which resulted in complete cure.

CONCLUSION

The microbiological profile of pyoderma reveals that Staphylococcus aureus is the commonest organism causing pyoderma. Among the *Staphylococcus aureus isolated* there were 13.6% of *methicillin resistant Staphylococcus aureus*. All of them showed a recurrence of infection since the empirical therapy was ampicillin - cloxacillin combination which is not effective. This inappropriate usage of antibiotics can lead on to emergence of resistance to higher antibiotics also. There was 51 isolates of Streptococcus which was the second common causative agent. Since there is a probability of going into complications like post streptococcal glomerulonephritis especially in children, isolation of Streptococcus is of prognostic significance.

Another important finding is that 38 cases showed mixed infections including few gram negative organisms. This high lights the fact that pus culture and sensitivity is mandatory for pyoderma cases before initializing antibiotic therapy.

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