Original Research Article Pyogenic skin infections by Gram Negative Rods- carbapenems are still the saviors?? Divya Shekokar^{1*}, Nitin Arun Ambhore², Rupali Satyen Mantri³

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Abstract

Introduction- Pyoderma is defined as "any pyogenic infection of the skin". Pyodermas are classified as primary or secondary and superficial or deep. Staphylcooccus aureus, Streptococcus pyogens, Escherichia coli, Klebsiella species, Pseudomonas aeruginosa, Acinetobacter species are common causes of pyoderma. Culture still remains the mainstay of diagnosis. Antibiotic susceptibility testing(AST) should be performed and reported to avoid unnecessary use of drugs. Aims and objectives- The objectives of the study were- 1. To isolate and identify the causative agents of pyodermas.2.To determine the antimicrobial profile of isolated Gram negative bacterias. Material and methods- 400 samples were collected from patients presenting with pyogenic skin infections attending Out Patient Department (OPD) and admitted in wards. The samples were transported in Cary Blair medium. Gram staining was done and culture was done on blood agar, MacConkey Agar and Nutrient Agar. Antibiotic susceptibility testing was performed according to Kirby Bauer disk diffusion method. Results- Out of 400 clinically suspected cases of pyoderma, male:female ratio was 1.16:1. 21-40 years age group was more commonly affected in both the sexes. Out of 400 samples, growth was found in 85.5% cases and no growth was there in 14.5% cases. Gram positive isolates were 266(74.93%) and Gram negative isolates were 89 (25.07%). Amongst Gram negative bacteria, Escherichia coli, Klebsiella species, Pseudomonas aeruginosa, Acinetobacter species were common causes. Primary pyoderma was more common(60.25%) cases. We found that, most of the Gram negative bacteria were sensitive to carbapenems and piperacillin and tazobactam. Resistance was noted to Amoxicillin and clavulanic acid, cefotaxime and ceftazidime. Conclusion- Gram Negative Bacteria are less common cause of pyoderma as compared to Gram positive bacteria. They are still sensitive to combination of piperacillin and tazobactam as well as carbapenems which indicates that, Antibiotic susceptibility testing should be performed and higher antibiotics like colistin should not be started immediately.

Keywords: Carbapenems, Escherichia coli, Piperacillin Tazobactam, pyoderma.

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Introduction

Pyoderma is defined as any purulent skin disease[1,2]. It is generally caused by one of the endogenic or exogenic pyogenic bacteria[3]. Primary pyoderma have a characteristic morphology caused, by a single organism, and arise in normal skin[4].Secondary pyoderma arise in a previously diseased skin as a superimposed condition[5] Gram negative rods are common causes of secondary pyoderma as compared to Gram positive bacteria. Common amongst them are-*Escherichia coli, Klebsiella species, Enterobacter species, Citrobacter species* and non fermentors like *Pseudomonas species* and *Acinetobacter* spp[6] Diagnosis is based on Gram stain and culture still remains the mainstay of diagnosis. Identification is further based on biochemical reactions[7]. For the successful treatment of pyodermas, knowing the sensitivity pattern and performing AST is must[8]

Aims and objectives

1) To isolate and identify the causative agents of pyodermas.

2) To determine the antimicrobial profile of isolated Gram negative bacterias.

Material and Methods

Study Design: Cross sectional prospective study.

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Study Period: 1st October 2016 to 30th September 2018.

Sample size: 400 samples from patients having pyoderma were collected and included in study. The sample size for the study was calculated using Open Epi Info Software Version 3.01.

Inclusion Criteria:Patients presenting to OPD and IPD with pyogenic skin lesions.

Exclusion Criteria:

- 1. Cases of resolving pyodermas not having active purulent discharge.
- 2. Patients not willing to participate in the study.

Ethical issues: Permission/consent in written to carry out the study was obtained from- Institutional Ethical committee of Medical College, Department of Microbiology and Patients.

Pus samples were collected and were transported in Cary Blair medium. It was followed by-

- 1) Gram staining
- 2) Bacterial culture mainly on Blood Agar, MacConkey agar and Nutrient agar.
- 3) Identification of pathogens
- Antimicrobial susceptibility testing of pathogens(CLSI guidelines)by Kirby Bauer disk diffusion method[9].

Results- A total of 400 pus samples from the patients clinically diagnosed with pyogenic skin infections of all age groups and both sexes either attending the dermatology OPD or admitted in the skin ward of Government Medical College, Akola were collected and studied for the bacteriological profile and antimicrobial susceptibility pattern from 1st October 2016 to 30th September 2018.

Out of 400 pus samples, 215(53.75%) samples were collected from male patients and rest 185(46.25%) from female patients. Male to

female ratio being 1.16:1. In the present study, out of the total 215 pus samples collected from male patients with pyogenic skin infections, maximum number of cases belonged to 21-40yrs age group constituting to a total of 53.04% followed by 41-60yrs age group accounting for 23.72% and 1-20yrs age group accounting for 14.41% and least number of samples were obtained from the above 60 years age group constituting 8.83%. Of the total 185 pus samples collected from the female patients with pyogenic skin infections, maximum number of cases belonged to 21-40yrs age group constituting to a total of 57.84% followed by 41-60years accounting for 23.78% and 1-20yrs age group with 10.82% and least number of patients belong to above 60 years age group constituting 7.56%.

Table 1: Culture results of the pus samples from the patients of pyogenic skin infections				
Yield of Samples	No. of cases	Percentage%		
Growth	342	85.5%		
No Growth	58	14.5%		
Total	400	100		

(Z test P value < 0.05 Significant)



Fig 1: Agewise and sexwise distribution among the culture positive cases

In this study, primary pyoderma was more common than secondary pyoderma constituting for 60.25% cases and 39.75% cases respectively.A total of 355 organisms were obtained from the culture of the samples from the patients of pyogenic skin infections, of which maximum organisms were gram positive which were 266 in number accounting for 74.93% and gram negative organisms were 89 in number accounting for 25.07%.

Table 2: Bacteriological analysis of the pus samples			
Organisms isolated	No. of cases(n=355)	Percentage (%)	
MRSA	193	54.36%	
MSSA	53	14.93%	
Streptococcus species	9	2.54%	
Enterococcus species	6	1.69%	
MRCONS	5	1.40%	
Escherichia coli	27	7.60%	
Klebsiella species and Klebsiella pneumonia	25	7.05%	
Proteus species(Proteus vulgaris and Proteus mirabilis	6	1.69%	
Pseudomonas species and Pseudomonas aeruginosa	18	5.07%	
Citrobacter species	7	1.97%	
Acinetobacter species	3	0.85%	
Enterobacter species	3	0.85%	
Total	355	100%	





Fig 2: Antibiotic sensitivity of the Enterobacteriaceae family

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In *Enterobacteriace*ae family, maximum sensitivity was observed for Meropenem, Imipenem, followed by Piperacillin – Tazobactam and

Tobramycin in the present study and maximum resistance was observed for Amoxycillin clavulanate, Ceftazidime and Cefotaxime.

Tabl	e 3: Antibiotic sensitivity	of Pseudom	onas species	(n=18)	

Drugs	Sensitive	Resistant
Ciprofloxacin	10	7
Gentamycin	12	6
Ceftazidime	4	14
Amikacin	14	4
Tobramycin	16	2
Aztreonam	13	3
Cefepime	4	12
Piperacillin	14	3
Piperacillin- Tazobactam	16	2
Imipenem	17	1
Meropenem	18	0

In pseudomonas maximum sensitivity was seen for Imipenem and Meropenem, followed by Piperacillin-Tazobactam and Tobramycin.

 Table 4: Antibiotic Sensitivity of Acinetobacter species (n=3)

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Drugs	Sensitive	Resistant		
Ampicillin sulbactam	2	1		
Cefotaxime	1	2		
Ciprofloxacin	1	2		
Tetracycline	2	1		
Gentamycin	2	1		
Cefepime	1	2		
Amikacin	2	1		
Tobramycin	2	1		
Piperacillin	2	1		
PiperacillinTazobactam	3	0		
Imipenem	3	0		
Meropenem	3	0		

Acinetobacter had maximum sensitivity was observed for Imipenem, Meropenem and Piperacillin- Tazobactam.

Discussion

This study was conducted as a thesis topic on 400 samples for 2 years.

In our study, male to female ratio was 1.16:1 in clinically suspected pyoderma cases. Similar findings were observed by Baslas et al (males 64.7% and females 35.3%) and Paudelet al[11] (males 65.3%) and females 34.7%) whereas the studies by Neirita Hazarika[12] showed female preponderance (52% females and males 48%) and also by S. Mariette Mathew et al[13] showed female preponderance (girls-60% and boys-40%). The more number of cases in males could be due to the fact that, outdoor work in males so they are more prone to trauma also in developing countries like India, health issues of females are ignored in many cases. In the present study, we observed that maximum number of patients belonged to 21-40 years age group showing males 53.04%(n=114) and females 57.84%(n=107) contributing to a total of 55.25% (n=221) similar to the study carried out by Anuja Gupta et al (48%)[6]and Hulmani et al.(21%)[14]. Whereas studies by CRV Narasimhalu et al[15]and Namratha Nandihal et al[16] showed that maximum number of pyodermal cases were from below 10 years age group. The reason for the occurrence of high incidence of pyoderma in the 2^{nd} decade of life may be consequent to more active life with games, work etc[17] Higher incidence in children could be due to increase in physical trauma in young age while playing, less awareness about hygiene and less immunity.Out of the total 400 samples, it was observed that 342(85.5%) were culture positive and 58(14.5%) were culture negative correlating with the study by Suresh Malhotra et al and Baslas et al showing culture positivity in 85.24% and 85.08% and culture negativity in 14.75% and 14.91% respectively. Study by Bhaskaran et al have showed culture negativity in 29.2% cases which was very high as compared to the present study. In our study, Gram

positive bacteria outnumbered Gram Negative bacteria which was in concordance with the study by Anuja Gupta et al[6] showing gram positivity in 76% patients and gram negativity in 24% patients. According to the study by Amarjeet Singh et al, gram positivity was seen in 94 % and gram negativity in 6 % samples respectively[4].In the present study, of all the gram negative isolates, Escherichia coli was the predominant pathogen similar to the study by Ajit Singh et al [20] and ChandanAshokan et al [17]. While study by Ashok Khare et al[21] and Anuja Gupta et al[6] reported Klebsiella pneumoniae to be the predominant pathogen among the gram negative isolates. In the present study primary pyoderma (60.25%) was more common than secondary pyoderma (39.75%) similar to the study by Prateek Kamble et al and ShankerVenkatesh et al.Few studies have shown almost similar number of primary and secondary pyodermas. The reason behind this may be due to timely management of primary skin disorders and traumas[7,2211]. Amongst the gram negative isolates in all bacterias E.coli, Klebsiella, Pseudomonas, Enterobacter, Citrobacter. Acinetobacter, maximum sensitivity was observed for Imipenem, Meropenem and Piperacillin- Tazobactam and maximum resistance was observed for Amoxycillin-clavulanate and Ceftazidime in the present study. In study of N.Singh et al and Ruturaj, Gram negative bacteria were found sensitive to Piperacillin and tazobactum[22,23].Carbapenems were not tested in the study of N. Singh et al. In the study of Harshita et al, carbapenems were 100 percent sensitive and most of the Gram negative bacteria were sensitive to PiperacillinTazobactum also which is comparable to our study[10,11,18,24]

Conclusion

Inour study we found that, cases of pyoderma were more common in males as compared to females. It could be due to prevalent male working population or ignorance of females to go to hospital and get checked. Primary pyodermas were more common as compared to secondary pyodermas. Gram positive bacteria outnumbered Gram negative bacterias. Amongst GNB, *E. coli* was most common. All Gram negative bacterias showed good sensitivity to Meropenem, Imipenem, Piperacillin and Tazobactam. This shows, Antibiotic Susceptibility testing should be done and higher antibiotics like colistin should not be started immediately.

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