

BACTERIOLOGICAL STUDY OF PYODERMAJ. K. Surekha¹, Syeda Amtul Moqueeth², M. L. Shashirekha³, M. Lingamurthy⁴, T. Sunitha⁵**HOW TO CITE THIS ARTICLE:**

J. K. Surekha, Syeda Amtul Moqueeth, M. L. Shashirekha, M. Lingamurthy, T. Sunitha. "Bacteriological Study of Pyoderma". Journal of Evolution of Medical and Dental Sciences 2015; Vol. 4, Issue 22, March 16; Page: 3845-3853, DOI: 10.14260/jemds/2015/553

ABSTRACT: AIM OF THE STUDY: To isolate the bacteria associated with pyodermas and to characterize the major isolate *Staphylococcus aureus* and to identify the MRSA. **MATERIAL & METHODS:** Study was conducted on 100 patients of pyodermas of all age groups and sex. Specimens were processed by performing microscopy, culture, biochemical reaction, antibiotic susceptibility, and the major isolate *Staphylococcus aureus* was further screened for Methicillin Resistant *Staphylococcus aureus*. **RESULTS:** In total 100 patients fulfilling the study criteria were evaluated. Impetigo (30%) was the most common clinical type followed by folliculitis (22%), showing male preponderance. The predominant isolate was *Staphylococcus aureus* which was further characterized for MRSA. Out of 69 cases 5 strains were identified as MRSA. **CONCLUSION:** Among the 100 samples processed bacteria isolated were *Staphylococcus aureus*, beta haemolytic streptococci and a mixture of *Staphylococcus* and *Streptococcus*. Predominant organism causing pyoderma was *Staphylococcus aureus*. Present study underline the need to take efforts to develop methods for rapid detection of MRSA to prevent Hospital Acquired Infections.

KEYWORDS: Pyodermas, *Staphylococcus aureus*, *Streptococcus pyogenes*, MRSA.

INTRODUCTION: Pyodermas one of the commonest clinical conditions is defined as pyogenic infection of the skin and its appendages.⁽¹⁾ Various factors⁽²⁾ contributory being-poverty, malnutrition, overcrowding, poor hygiene, low socio-economic strata, climatic conditions also play a key role with hot and rainy season being the period of maximum occurrence.

Pyodermal infections are mainly due to *Staphylococci* and *Streptococci* strains,⁽³⁾ *Staphylococcus aureus* being responsible for 18% of all Nosocomial infections, causes 1/3 of post operative skin infections. Infections caused by *Staphylococcus aureus* used to respond to beta lactum group of drugs. However due to increased incidence of penicillin and methicillin resistant strains of *Staphylococcus aureus*,^(4,5) treatment of these infections have become problematic. Changing trends being noted in the etiologic agent of pyodermas and with the advent of drug resistance it is essential to know the correct etiology, antibiotic sensitivity pattern for treatment purpose and to prevent the feared sequelae

The present study was conducted on an attempt to study the incidence frequency antibiotic sensitivity pattern of the most prevalent organism *Staphylococcus aureus* isolated from pyoderma cases attending Department of Dermatology, Osmania General Hospital, Hyderabad.

MATERIAL AND METHODS:

MICROSCOPY AND BACTERIAL CULTURES: Isolation of the organisms was based on morphology cultural and biochemical characteristic. Antibiotic sensitivity was done and MRSA strains identified. Specimen collected was pus from the skin lesions in the form of swab. Three swabs were collected. One swab was used to prepare smear and Gram stain performed to determine the likely organism

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present. Second swab was inoculated on to the culture media-nutrient Agar, blood Agar, Mackonkey Agar and incubation for 24 hrs at 37°C. Third swab was used to inoculate in glucose broth incubated at 37°C for 24 hrs then inoculated onto solid media-for isolating streptococcus.

Smear made from colonies grown on the culture media, Gram stain was performed and observed under microscope-violet coloured spherical cocci approximately 1 μ in diameter arranged characteristically in grape like clusters-identified as Staphylococcus.

The colonies were further sub-cultured into nutrient broth incubated at 37°C for 2 hrs, uniform turbidity was observed indicating growth of the organism. This subculture was used to perform the various biochemical tests and inoculated on various selective media to identify Staphylococcus aureus as follows.

SELECTIVE MEDIA: Milk agar, High salt agar, Mannitol salt agar, Potassium Tellurite agar.

BIOCHEMICAL TESTS: Catalase test, Coagulase test, Nitrate reduction test, Gelatin liquefaction, Indole test, Methyl Red test, Voges Proskauer test, Urease test, Phosphotase test, Sugar fermentation test, Hughs-Leifson's oxidation-fermentation test.

ANTIBIOTIC SUSCEPTIBILITY TESTING: Susceptibility and MICs⁽⁶⁾ were determined by disc diffusion method using Muller Hinton agar, following the CSLI guidelines. Antibiotic discs were commercially obtained. Antibiotics used were Penicillin G (10 units), Methicillin (5 μ g), Erythromycin (5 μ g), Gentamycin (10 μ g), Amikacin (30 μ g), Cephalexin (30 μ g), Ciprofloxacin (5 μ g), vancomycin (30 μ g).

Methicillin resistance⁽⁵⁾ was demonstrated by using,

1. Nutrient agar with 6% sodium chloride-incubated at 37°C for 24 hrs.
2. Muller Hinton agar-incubated at 30°C for 40 hrs.

SELECTIVE MEDIA USED FOR MRSA DETECTION:

1. Mannitol salt agar-to which is added 6mg/l oxacillin or 10mg/l of methicillin.
2. Methicillin agar-Muller Hinton agar containing 4% sodium chloride and 6mg/l oxacillin or 10mg/l methicillin.

These plates were streaked by broth culture incubated at 35°C for 24 hrs-growth was observed with MRSA.

IDENTIFICATION OF STREPTOCOCCI:

MORPHOLOGY: Smear prepared, Gram staining done from the colonies and observed under the microscope-violet colour spherical cocci arranged in chains were identified as Streptococci. Subculture was done in glucose broth and various biochemical tests performed-Catalase test, bile solubility test, sugar fermentation test, Hydrolysis of Pyrothidonyl- β -naphthylamide (PYR test), Bacitracin sensitivity, and antibiotic susceptibility.

RESULTS: Pyoderma clinical types⁽⁷⁾ incidence - Of the 100 cases studied Impetigo 30% was the most commonest clinical type followed by Folliculitis (22%), Furunculosis (10%), Ecthyma (6%),

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Infected scabies (5%), Sycosisbarbae (5%), Erythrasma (5%), Cellulitis (3%), Infectious Eczematous Dermatitis (3%), Paronychia (3%), Carbuncle (2%), Periporitis (2%), Infected wound (2%), Job's syndrome (1%), Pitted keratolysis (1%).

Sl. No	Types of Pyodermas	No. of Cases	Percentage
1.	Impetigo Contagiosum	20	20%
2.	Bullous Impetigo	10	10%
3.	Ecthyma	6	6%
4.	Folliculitis	22	22%
5.	Furunculosis	10	10%
6.	Carbuncle	2	2%
7.	Sycosisbarbae	5	5%
8.	Cellulitis	3	3%
9.	Job's Syndrome	1	1%
10.	Acute Paronychia	3	3%
11.	Erythrasma	5	5%
12.	Pitted Keratolysis	1	1%
13.	Periporitis	2	2%
14.	Infected Scabies	5	5%
15.	Infected wound	2	2%
16.	Infected Eczematous Dermatitis	3	3%

TABLE 1: TYPES OF PYODERMAS

ANALYSIS OF PYODERMAS: Of the 30 cases of impetigo - 21(70%) isolated were Coagulase Positive Staphylococcus, 5 isolates (16.66%) were Streptococcus pyogenes, 4 isolates (13.33%) were mixed with Staphylococcus and Streptococcus pyogenes.

Bacteriological analysis: Of the 100 samples processed, 96 samples yielded growth, 4 samples did not show any bacterial growth.

No. of Samples	No. of samples Which organisms were isolated	Single organism isolated	Mixture of Organisms Isolated	Samples in which no organisms were Isolated
100	96(96%)	82(82%)	14(14%)	4(4%)

TABLE 2: BACTERIOLOGICAL ANALYSIS

Types of bacteria isolated-Of the 96 samples, 82 samples showed single organism, 14 samples showed mixed organisms, out of the 82 samples 69 were isolated as coagulase positive Staphylococcus, 13 were isolated as β -haemolytic Streptococci, 14 samples showed mixed growth with Staphylococcus and Streptococcus. The predominant etiological agent was Staphylococcus aureus.

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No. of Samples	No. of samples which organisms were isolated	Single organism isolated	Mixture of Organisms Isolated	Samples in which no organisms were Isolated
100	96(96%)	82(82%)	14(14%)	4(4%)

TABLE 3: TYPES OF ORGANISMS ISOLATED

The sex distribution showed male preponderance of 58 cases (58%) and females 42 cases (42%) with male, female ratio being 58:42. In age distribution pyoderma occurred most frequently among 1 to 10 year age group (37%) followed by (18%) in 21-30 years (15%) cases in 11-20 years, (10%) each in 31-40 years 41-50 years age group, (5%) in 51-60 years age group, occurred less commonly in age group 61-70 (3%) and 71-80 years (2%).

Age Group	No. of Cases		Total
	Males	Female	
1-10 years	22	15	37
11-20 years	9	6	15
21-30 years	8	10	18
31-40 years	6	4	10
41-50 years	5	5	10
51-60 years	3	2	5
61-70 years	3	Nil	3
71-80 years	2	Nil	2
Total	58	42	100

TABLE 4: SEX DISTRIBUTION IN RELATION TO AGE

Of the 22 cases^(7,8) of Folliculitis 19 (86.36%) were Staphylococcus aureus, 3 isolates (13.63%) were mixed. Out of 10 cases of Furunculosis 6 isolates (60%) were Staphylococcus aureus, 4 isolates (40%) were Streptococcus pyogenes. Of the 6 cases of Ecthyma 1 isolate (16.6%) was Staphylococcus aureus, 4 isolate (66.66%) was Streptococcus pyogenes, 1 isolate (16.66%) was mixed. Erythrasma-5 cases (100%) were Staphylococcus aureus. Sycosis Barbae - 5 cases (100%) were Staphylococcus aureus. Infected scabies - Of the 5 cases 3 isolates (60%) were Staphylococcus aureus, 2 isolates (40%) were mixed. Of the 3 cases of cellulites 1 isolate (33.33%) was mixed, 2 cases (66.66%) showed no bacterial growth. Acute Paronychia - 3 cases (100%) were Staphylococcus aureus. Of the 3 cases of Infected Eczematous dermatitis 2 isolates (66.66%) were mixed 1 case (33.33%) showed no bacterial growth. Of the 2 cases of Carbuncle 2 isolates (100%) Staphylococcus aureus. Periporitis-2 isolates (100%) were Staphylococcus aureus. Of 2 cases of infected wound 1 isolate (50%) was Staphylococcus aureus, 1 isolate (50%) was mixed. Of the 1 (100%) case of Job's Syndrome no bacterial growth was seen.

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Types of Pyoderma	Total No. of cases	Coagulase positive Staphylococci	Beta-haemolytic Streptococci	Mixed both Staphylococci Streptococci	No. bacterial growth
Impetigo contagiosa	20	12(60%)	5(25%)	3(15%)	-
Bullous impetigo	10	9(90%)	-	1(10%)	-
Ecthyma	6	1(16.6%)	4(66.6%)	1(16.6%)	-
Folliculitis	22	19(86.36%)	-	3(13.6%)	-
Carbuncle	2	2(100%)	-	-	-
Sycosisbarbae	5	5(100%)	-	-	-
Furunculosis	10	6(60%)	4(40%)	-	-
Cellulites	3	-	-	1(33.33%)	2(66.66%)
Job's Syndrome	1	-	-	-	1(100%)
Acute Paronychia	3	3(100%)	-	-	-
Erythrasma	5	5(100%)	-	-	-
Pitted Keratolysis	1	1(100%)	-	-	-
Periporitis	2	2(100%)	-	-	-
Infected Scabies	5	3(60%)	-	2(40%)	-
Infected Wound	2	1(50%)	-	1(50%)	-
Infected Eczematous Dermatitis	3	-	-	2(66.66%)	1(33.33%)
Total	100	69	13	14	4

TABLE 5: ORGANISMS ISOLATED FROM DIFFERENT OF PYODERMAS

Bacterial analysis from individual pyodermas- On analysis of *Staphylococcus aureus* isolated from individual pyoderma-Impetigo 21 isolates (70%), Folliculitis 19 isolates (86.36%), Furunculosis 6 isolates (60%), SycosisBarbae 5 isolates (100%), Erythrasma 5 isolates (100%), Acute paronychia 3 isolates (100%), infected scabies 3 isolates (60%), Carbuncle 2 isolates (100%), Periporitis 2 isolates (100%), Infected wound 1 isolate (50%) pictedkeratolysis 1 isolate (100%).

On analysis *Streptococcus pyogenes*⁽⁹⁾ - Impetigo 5 isolates (16.66%), Ecthyma 4 isolates (66.66%), Furunculosis 4 isolates (40%).

Sl. No.	Types of Pyodermas	No. of Strains Isolated	Percentage
1.	Impetigo Contagiosa	12	60%
2.	Bullous Impetigo	9	90%
3.	Ecthyma	1	16.66%
4.	Folliculitis	19	86.36%

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5.	Carbuncle	2	100%
6.	SycosisBarbae	5	100%
7.	Furunculosis	6	60%
8.	Acute Paronychia	3	100%
9.	Erythrasma	5	100%
10.	Pitted Keratolysis	1	100%
11.	Periporitis	2	100%
12.	Infected Scabies	3	60%
13.	Infected Wound	1	50%

TABLE 6: STAPHYLOCOCCUS AUREUS ISOLATED FROM INDIVIDUAL PYODERMA

Sl. No.	Types of Pyodermas	No. of Strains Isolated	Percentage
1.	Impetigo	5	16.66%
2.	Ecthyma	4	66.66%
3.	Furunculosis	4	40%

TABLE 7: STREPTOCOCCUS PYOGENES ISOLATED FROM INDIVIDUAL PYODERMA

Antibiotic profile of Staphylococcus aureus- Highest susceptibility to Cefotaxime (84.06%), Cephalexin (82.3%), followed by Erythromycin (75.37%), Tetracycline (62.9%), Ciprofloxacin (50.73%), Gentamycin (44.93%), Amikacin (42.03%), least susceptibility to Penicillin (18.18%) and Ampicillin (22.04%) was observed.

Antibiotic profile of Streptococcus pyogenes - Highest susceptibility to Cefotaxime (96.2%), Cephalexin (95%), followed by Erythromycin (92.3%), Ciprofloxacin (85.2%), Gentamycin (80.2%), Tetracycline (78.3%), Ampicillin (70.8%), Penicillin (65.2%), and least susceptibility to Amikacin (52.63%) was observed.

Antibiotic	Coagulase Positive Staphylococci	Beta-Haemolytic Streptococci
Penicillin	18.18%	62.96%
Ampicillin	21.73%	70.37%
Erythromycin	75.37%	92.59%
Gentamycin	44.93%	77.77%
Cefotaxime	86.95%	96.29%
Cephalexin	84.06%	88.88%
Ciprofloxacin	50.73%	85.18%
Amikacin	42.03%	51.85%
Tetracycline	62.31%	81.48%

TABLE 8: SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCUS AUREUS AND STREPTOCOCCUS PYOGENES

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Antibiotic resistance profile of *Staphylococcus aureus*- showed highest resistance towards Penicillin (81.15%), followed by Amikacin (57.97%), Gentamycin (55.07%), Ciprofloxacin (49.27%), Erythromycin (24.63%), Cephalexin (15.94%), Methicillin (7.24%), and Vancomycin (2.81%).

MRSA: Out of the 69 cases of *Staphylococcus aureus* only 5 strains showed resistance.

Sl. No	Name of the Antibiotic	No. of Resistant Isolates	Percentage
1.	Penicillin	56	81.15%
2.	Amikacin	40	57.97%
3.	Gentamycin	38	55.07%
4.	Ciprofloxacin	34	49.27%
5.	Erythromycin	17	24.63%
6.	Cephalexin	11	15.94%
7.	Methicillin	5	7.24%
8.	Vancomycin	2	2.89%

TABLE 9: RESISTANCE PATTERN OF ISOLATES OF STAPHYLOCOCCUS AUREUS

DISCUSSION: Of the 100 samples of pus collected from cases of pyodermas, 96 cases yield growth, out of which 69 were *Staphylococcus aureus*, 13 were *Streptococcus pyogenes*, 14 were mixture of *Staphylococcus aureus* and *Streptococcus pyogenes* and 4 did not yield any organism. These findings were in agreement with various other workers. According to Parischa⁽¹⁰⁾ A Bhujwala R.A. Srinivas (1972)-in their study revealed the incidence of organisms as (68%) *Staphylococcus aureus*, 5% *Streptococcus pyogenes* and 17% both the organisms. In the present study also the predominant organism was *Staphylococcus aureus*.

Kishore J. Singh, O.P. Gupta (1989) ⁽¹¹⁾ in their study bacteriology of pyoderma showed out of 100 samples, 99 yielded positive cultures *Staphylococcus aureus* alone (45%) and *Streptococcus pyogenes* was (32%) the commonest isolates. According to Singh G. Bhattacharya K in a study on bacteriology of pyodermas (1989), *Staphylococcus aureus* was isolated from 109 (61.93%) beta-haemolytic *Streptococci* from 22(12.5%) and both these organism from 29(16.48%) cases out of the 176 cases

In an analysis of 127 patients with pyoderma by Lee CT, Tay L. (1990)-38 (30%) had primary pyodermas and 89 (70%) had secondary pyodermas of the primary pyodermas (71%) were due *Staphylococcus aureus* and 5% were due to multiple organism. Of the secondary pyodermas 44% were due to *Staphylococcus aureus* and 21% were due to *Streptococci*. Adarsh Chopra et al (1994) isolated 93.78% *Staphylococcus aureus*, 7.07% *Streptococcus pyogenes* and 7.07% both the organisms.

In the clinico-bacteriological study of pyodermas in children by Kaker N, Kumar V, et al (1999) primary pyodermas were observed in 72% of the children and secondary pyodermas in (28%). Impetigo was the commonest primary pyoderma (48.6%) and infected scabies in secondary pyoderma (42.86%). Single organism was isolated from majority (84%) of which *Staphylococcus aureus* was 48% and beta-haemolytic *Streptococcus* was (36%), a combination of both was observed from (16%). Prospective study was conducted on patients with pyoderma among Hajj pilgrims in

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Makkah (2002). Of the 80 cases (52.5%) were primary, (45.7%) were secondary. Impetigo being the predominant lesion in primary (28.8%) and infected eczema in secondary pyodermas (18.8%). The organisms isolated were in the primary pyoderma *Staphylococcus aureus* (65.6%), *Streptococcus pyogenes* (28.1%) both (6.4%). In secondary pyodermas *Staphylococcus aureus* (44.7%) *Streptococcus pyogenes* (15.8%), both (18.4%).

The *Staphylococcus aureus* isolated in the present study showed highest resistance to penicillin (81.15%) which was in accordance with the studied of Singh G. Bhattacharya K. (1989), Kakar N,⁽¹²⁾ Kumar V et al (1999) (85.9%), Fatani MI et al⁽¹³⁾ 2002 (80-85%) whereas earlier studies showed a low resistance pattern Parikh⁽¹⁴⁾ D.A et al-1987 (49%) and Ramani et al (1980) (4%). The present study indicated that epidemiology of MRSA is also changing over the past few decades. In this study the incidence of MRSA was found to be 31.88%, in accordance with Nishijima et al 1995 showed 20-40% resistance, Brig V.C. Ohri 1988 showed 33.6%, Brig V.C. Ohri (1992) showed 34.2% resistance.

CONCLUSION: In the present study bacteriological study of pyodermas of the 100 samples processed the organisms isolated were *Staphylococcus aureus*, beta-haemolytic *Streptococci* and a mixture of *Staphylococcus aureus* and beta-haemolytic *Streptococci*. The predominant organism isolated was *Staphylococcus aureus* (69%). There was no organism isolated from 4 samples probably due to treatment taken by the patient. Most of the cases occurred in the low age group 1 to 10 years. There was female preponderance. Most of the cases were isolated from low socio-economic group. Of the 100 cases of pyodermas (90%) were primary and 10% were secondary Impetigo, Folliculitis and Furunculosis were common among the primary pyodermas. Infected scabies was common among secondary pyodermas. In the antibiotic susceptibility testing of *Staphylococcus aureus* most of the strains were found to be susceptible to Cefotaxime, Cephalexin, Erythromycin and Ciprofloxacin. Maximum resistance was observed towards penicillin. *Streptococcus pyogenes* was found to be susceptible to most of the antibiotics with highest sensitivity to Cefotaxime and Erythromycin.

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Date of Submission: 06/02/2015.
Date of Peer Review: 07/02/2015.
Date of Acceptance: 04/03/2015.
Date of Publishing: 14/03/2015.