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Bacteriological Profile of Necrotising Fasciitis in A Teritary Care Centre

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ABSTRACT
Necrotising fasciitis is a potentially fatal infection that is rapidly progressive involving widespread necrosis of superficial fascia and subcutaneous tissue. It is usually associated with diabetes mellitus, trauma, Cirrhosis liver, chronic kidney disease, etc. The present study was conducted in the Department of Microbiology, Govt. Medical College, Thiruvananthapuram for a period of 1 year from May 2014 to April 2015. The objective of the study was to isolate and identify the bacterial pathogens causing Necrotising fasciitis from clinically diagnosed cases and to determine the Antibiotic susceptibility pattern of the isolates. A total number of 235 samples collected from the lesions of patients with necrotizing fasciitis admitted in the surgery wards during this period. Culture was positive in 222 samples (94.5%). Out of the total isolates, 96.5% were monomicrobial and 3.5% were poly microbial. The predominant isolate in the study was pseudomonas aeruginosa (37.44%) other isolates are Staphylococcus aureus (19.1%), Klebsiella species (11.91%), E.coli (7.65%), Streptococcus pyogenes (4.25%), Enterococci & Proteus vulgaris (3.4% each), Proteus mirabilis & CONS (2.97% each), MRSA (1.7%) and Enterobacter species (0.42%). The antibiotic sensitivity pattern of pseudomonas aeruginosa showed 100% sensitivity to Amikacin, Ceforazone – Sulbactum and Piperacillin tazobactam. Staphylococcus aureus isolates were sensitive to Cloxacilin, Amikacin and Vancomycin (100%). All the strains were resistant to penicillin. After treatment with appropriate antibiotics according to the antibiotic sensitivity pattern and surgical intervention, 204 (86.8%) patients survived. Mortality rate was 13.2% which was significantly reduced when compared to other studies because of the early treatment with appropriate antibiotics.
Keywords: *Necrotising fasciitis, antibiotic susceptibility testing.*

Introduction

Necrotising fasciitis is a life threatening soft tissue infection primarily involving superficial facia. The term necrotising fasciitis was first proposed by Wilson in 1952. The anaerobic renaissance started in late 1960s. It was later in 1977 that Giuliani determined that necrotizing fasciitis was due to polymicrobial infections caused by a variety of microorganisms including aerobic and anaerobic gram positivecocci and gram negative bacilli. This disease had been known by other names such as necrotizing cellulitis, streptococcal gangrene, Meleney ulcer, phagedaenic ulcer, dermal gangrene and flesh-eating bacteria syndrome.

Necrotising fasciitis is usually precipitated by injury/trauma, post operative wound infection, burns ulcers, abscess, insect bites and human bites. Mortality rate without treatment approaches 100%. Even with treatment the mortality rates of this is has remained alarmingly high. The high morbidity and mortality associated with necrotizing fasciitis makes it both a medical and surgical emergency. Early diagnosis surgical intervention combined with administration of appropriate antibiotics is the cornerstone of this treatment. Prognosis depends on initiation of appropriate antibiotic treatment.

The aim of our study is to isolate and identify the bacterial pathogens causing necrotizing fasciitis from the clinical specimen collected from clinically diagnosed cases of necrotizing fasciitis and to determined the antibiotic sensitivity pattern of these isolates so that appropriate antibiotic treatment can be started at the early phase of the disease. There by the morbidity and mortality can be significantly reduced.

Materials and Methods

Patient and study design

Patients with age of 18 years and above were included in the study. Samples were collected from all clinically diagnosed cases of necrotizing fasciitis admitted in surgery wards at Govt. Medical College Hospital, Trivandrum, Kerala, India during the period of 1 year from May 2014 to April 2015. Culture & sensitivity of the isolates obtained from clinical samples were done in the department of Microbiology at Govt. Medical College, Trivandrum.

Study design – Descriptive study

Study size – all patients in that period

Collection of samples

Three different types of samples were collected from patients – soft tissue specimens, Exudate from ulcers and syringe aspirates from the lesion were collected under sterile precautions for the study purpose. Tissue samples were collected from the base of debrided ulcers using sterile scalpel blade. Exudates from ulcers were collected after thoroughly cleaning the site with sterile normal saline. Using two sterile swabs, the exudate from the lesion were collected where one was used for gram staining and other for doing culture. Pus samples were aspirated with sterile syringe and needle after cleaning the site with proper antiseptic. Specimens were transported immediately after collection and processed without any delay in the Central Microbiology Laboratory at Govt. Medical College Hospital, Thiruvananthapuram. 2 blood samples 5ml each were collected under aseptic precaution at an interval of half an hour from two different size by venepuncture from suspected cases of septicemia.

Processing of Samples

Immediately after collection of samples Gram staining was done to demonstrate the morphology and arrangement of the bacteria.

Aerobic culture

Each specimen was inoculated on blood agar, Mac Conkey agar, Manitol salt agar and glucose broth and incubated at 37^0C for aerobic culture.

Anaerobic culture

The sample was inoculated into Robertson's cooked meat medium and incubated at 37^0C for isolating anaerobic bacterium. Alkaline pyrogallol method was also used to isolate anaerobic bacteria in culture.

After doing Gram staining, if gram positive cocci in clusters are observed then Catalase test and Coagulase tests were done. If gram negative bacilli are seen, Catalase test, Oxidase test and relevant biochemical reaction were done for the identification of bacteria. Antibiotics sensitivity testing of the bacterial isolates were done on Mueller – Hinton agar using Kirby Bauer disc diffusion method.

Materials and Methods

Samples were collected from all patients satisfying the clinical criteria for Necrotizing Fasciitis who were admitted in surgery units S1 to S6, Government Medical College Hospital,

Thiruvananthapuram during the period of 1 year from May 2014 to April 2015. Total number of 235 samples was collected .Three different types of samples were collected from cases of Necrotizing Fasciitis. Soft tissue specimens, exudate from ulcers and syringe aspirates were collected under sterile precautions for the study purpose.

Collection of specimen: Tissue samples were taken from base of debrided ulcers using sterile scalpel blade. Exudates from ulcers were collected after thoroughly cleaning the site with sterile normal saline. Using two sterile swabs, the exudate from the lesion were collected where one was used for gram staining and other for doing culture. Pus samples were aspirated with sterile syringe and needle after cleaning the site with proper antiseptic. Specimens were transported as early as possible after collection and processed without any delay in the Central Microbiology Laboratory at Medical College Hospital. 2 blood samples 5ml each were collected under aseptic precautions, at an interval of half an hour from two different sites by venipuncture, from suspected cases of septicemia.

Processing of Samples

I Direct Microscopy

Morphology and arrangement of the microorganism was studied using Gram's staining method.

II Culture

Each specimen was inoculated on blood agar, MacConkey agar, Mannitol salt agar, Glucose broth for aerobic culture. Alkaline pyrogallol method and Robertson's cooked meat medium were the culture methods used for isolating anaerobic bacteria.

III. Antimicrobial sensitivity of the bacterial isolates was done on Mueller Hinton agar using Kirby Bauer disc diffusion method.

Results & Discussion

Three different types of samples were collected from the site of lesion from patients with Necrotising Fasciitis and blood sample was collected from patients suspected with septicaemia.

Table 1: Types of Samples

S.No	Nature Of Samples	Total Number	Percentage
1	Aspirated pus	125	53.20%
2	Exudate (double swab)	82	34.89%
3	Tissue	28	11.91%
	Total	235	100%

Table 2: Analysis of culture

Total No. Of Samples Collected	Total No. Of Isolates	Culture Positives	Culture Negatives
235	222	94.46 %	5.54%

Table 3: Sample analysis

S.No	Nature Of Samples	Total No.	Isolates	Percentage
1.	Aspirated Pus	125	123	98.4%
2.	Swabs	82	72	87.80%
3.	Tissue	28	27	96.43%
	Total	235	222	94.46%

Out of the total 235 samples collected from the lesions of patients with Necrotising Fasciitis, culture was positive in 222 samples (94.5%). Aspirated pus samples collected in this study was 125, of which the culture was positive in 123 samples (98.4%).The culture positivity was 96% in exudate and tissue. The aspirated pus is found to be the best specimen to isolate bacteria in pure culture. Aspirated pus samples yielded pure growth of monomicrobial (96.5%) and polymicrobial growth (3.5%).

Table 4: Blood sample analysis

Blood Samples				
Total No.	Culture Positives	Percentage	Culture Negatives	Percentage
10	3	30 %	7	70%

Blood samples were collected from 10 cases and the culture was positive in 3 cases (30%).

Table 5: Gender distribution

	Male:	Female:	Total No.
Total Number	203	32	235
Percentage	86.3%	13.7%	100%

Incidence was high in males showing upto 203 (86.3%) in number and females only 32(13.7%) in number.

Table 6: Age Distribution

Age Group In Years	Number	Percentage
Adults 18-20	1	0.42%
21-30	21	8.93%
31-40	10	4.25%
41-50	51	21.70%
51-60	59	25.10%
61-70	74	31.48%
71-80	16	6.80%
81-90	2	0.85%
91-100	1	0.42%
Total	235	100%

Total number of 235 samples was collected from patients with Necrotising Fasciitis of the age group ranging from 18 yrs to 92 yrs. The minimum age of patient from whom sample was obtained was 19 years and the maximum age was 92 years .The most common age group in which Necrotising Fasciitis was seen in our study in this institute is between 61 -70yrs (31.48%).

Table 7: Analysis of anatomical sites of lesion

Site	Number	Percentage
Lower Limbs	214	91%
Perineum	13	5.50%
Upper Limbs	4	1.70%
Abdomen	4	1.70%
Total	235	100%

The most common site of infection observed in our study is the lower limbs 91%. Next common site of infection is perineum (5.5%) followed by upper limbs and abdomen (1.7% respectively).

A patient with lower limb Necrotising Fasciitis

Table 8 :Analysis of predisposing factors

The most common risk factor identified in our study was Type 2 DM (40%) and the next common factor was age >60 yrs (32%) followed by trauma (13%), post-operative infection (3%) and Psoriasis (2%).

Microbiological culture 70% showed growth and 30% showed no growth.

Table 9 : Nature of bacterial growth pattern

	Polymicrobial	Monomicrobial	Total Isolates
Number	12	210	222
Percentage	5.4%	94.6%	100%

Among the polymicrobial organisms isolated from culture, combinations of *E.coli +Streptococcus pyogenes , Streptococcus pyogenes + Klebsiella , Pseudomonas + E.coli, Staphylococcus aureus +Klebsiella, Proteus mirabilis + E.coli, CoNS +Klebsiella, Pseudomonas + CoNS, Pseudomonas +Proteus vulgaris ,Klebsiella + CoNS* were obtained.

Among the monomicrobial culture positive cases, the organisms isolated are *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella species, E.coli, Streptococcus pyogenes, Enterococcus* and *Proteus vulgaris, Proteus mirabilis* and *CoNS, MRSA and Enterobacter*.

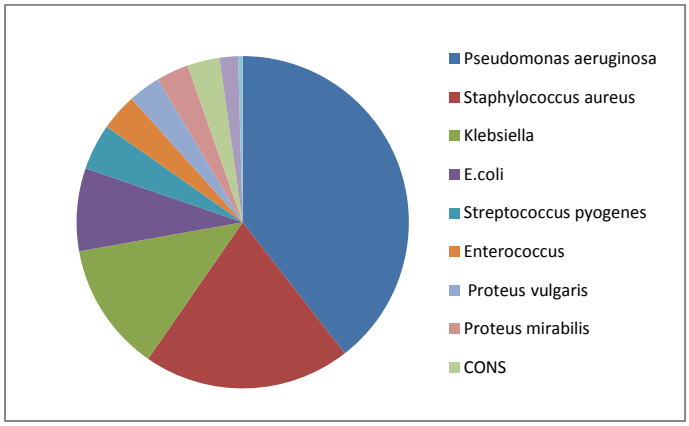

Table 10: Clinical isolates from the lesion

Isolates	Number	Percentage
Pseudomonas aeruginosa	88	37.44%
Staphylococcus aureus	45	19.1%
Klebsiella	28	11.91%
E.coli	18	7.65%
Streptococcus pyogenes	10	4.25%
Enterococcus	8	3.40%
Proteus vulgaris	8	3.40%
Proteus mirabilis	7	2.97%
CONS	7	2.97%
MRSA	4	1.70%
Enterobacter	1	0.42%
Total	222	100%

Among the total isolates, *Pseudomonas aeruginosa* is the predominant species identified (37.44%).

The other isolates obtained from the samples are *Staphylococcus aureus* (19.1%). *Klebsiella spp* (11.91 %), *E.coli* (7.65 %).

Next was *Streptococcus pyogenes* (4.25%) followed by *Enterococcus spp* and *Proteus vulgaris* (3.40% each), *Proteus mirabilis* and *CONS* (2.97% each), *MRSA* (1.70%) and *Enterobacter* (0.42%).

Antibiotic Sensitivity pattern of bacteria isolated from the lesion:

Cefoperazone-Sulbactam and Piperacillin-Tazobactam were the most commonly empirically administered antibiotic for treatment since most *Gram negative isolates* were sensitive to it.

On the other hand, Cloxacillin was the most common sensitive administered antibiotic for Gram positive *Staphylococcus aureus*.

Other antibiotics administered to which the isolates showed sensitivity were Erythromycin, Gentamicin, Amikacin, Ciprofloxacin, and 1st and 3rdgeneration Cephalosporins.

Clinical Isolates in the Study

Gram Positive Bacterial Isolates

Table13: Antibiotic Sensitivity Pattern of Gram positive isolates

The gram positive bacteria isolated in this study are *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus, Coagulase Negative Staphylococci (CoNS), and Methicillin Resistant Staphylococcus aureus (MRSA).*

Antibiotic Sensitivity Pattern of *Staphylococcus aureus:*

Total no. of cases = 45

Antibiotic	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin	0	45	0%	100%
Erythromycin	24	21	53.3%	46.7%
1st generation Cephalosporins	45	0	100%	0%
Gentamicin	42	3	93.3%	6.7%
Cloxacillin	45	0	100%	0%
Amikacin	45	0	100%	0%
Vancomycin	45	0	100%	0%

The antibiotic sensitivity pattern of *Staphylococcus aureus* obtained showed that the isolates were 100% sensitive to Cloxacillin, Amikacin, and Vancomycin. 95.6% of the isolates were sensitive to I[st] generation Cephalosporins and 4.4% strains were resistant. 93.3% were sensitive to Gentamicin and 6.7% were resistant. 53.3% were sensitive to Erythromycin and 46.7% were resistant. Strains were 100% resistant to Penicillin.

Antibiotic Sensitivity Pattern of *Streptococcus pyogenes*: (10 Isolates)

Antibiotic	No. Of Isolates	Percentage

	Sensitive	Resistant	Sensitive	Resistant
Penicillin	10	0	100%	0%
Ampicillin	7	3	70%	30%
Erythromycin	6	4	60%	40%
1st Generation Cephalosporins	8	2	80%	20%
Gentamicin	0	10	0%	100%

The antibiotic sensitivity pattern of *Streptococcus pyogenes* obtained showed that the isolates were 100% sensitive to Penicillin. 80% of the isolates were sensitive to 1st generation Cephalosporins and 20% strains were resistant. 70% were sensitive to Ampicillin and 30% were resistant. 60% were sensitive to Erythromycin and 40% were resistant. Strains were 100% resistant to Gentamicin.

Antibiotic Sensitivity Pattern Of *Enterococcus spp*: (8) isolates

Antibiotic	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin	0	8	0%	100%
Ampicillin	6	2	75%	25%
Erythromycin	2	6	25%	75%
Gentamicin	0	8	0%	100%

The antibiotic sensitivity pattern of *Enterococcus* obtained showed that the isolates were 100% resistant to Penicillin. 75% were sensitive to Ampicillin and 25% were resistant. 25% were sensitive to Erythromycin and 75% were resistant. All the strains were resistant to Gentamicin (100%).

Antibiotic Sensitivity Pattern of *Coagulase Negative Staphylococci (CoNS):*

Total no. of isolates = 7

Antibiotic	Sensitive	Resistant	Sensitive	Resistant
Penicillin	0	7	0%	100%
Erythromycin	0	7	0%	100%
Cephalosporin	2	5	28.6%	71.4%
Gentamicin	4	3	57.1%	42.9%
Cloxacillin	2	5	28.6%	71.4%
Amikacin	6	1	85.7%	14.3%
Vancomycin	7	0	100%	0%

The antibiotic sensitivity pattern of CoNS obtained showed that the isolates were 100% sensitive to Vancomycin. 85.7% of the isolates were sensitive to Amikacin and 14.3% strains were resistant. 57.1%were sensitive to Gentamicin and 42.9% were resistant. 28.6% were sensitive to 1st generation Cephalosporins and Cloxacillin respectively and 71.4% were resistant to each. Strains were 100% resistant to Penicillin and Erythromycin.

Antibiotic Susceptibility Pattern of *Methicillin Resistant Staphylococcus Aureus (MRSA):* 4 *Isolates*

Antibiotic	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Linezolid	3	1	75%	25%
Rifampicin	3	1	75%	25%
Amikacin	3	1	75%	25%
Vancomycin	4	0	100%	0%
Clindamycin	3	1	75%	25%

The antibiotic sensitivity pattern of *Methicillin Resistant Staphylococcus aureus (MRSA)*obtained showed that the isolates were 100% sensitive to Vancomycin. 75% were sensitive to Linezolid, Rifampicin, Amikacin and Clindamycin with 25% resistance to each of them respectively.

Gram Negative Bacterial isolates

The gram negative bacteria isolated in our study are *Pseudomonas aeruginosa, Klebsiella species, E.coli., Proteusvulgaris, Proteus mirabilis and Enterobacter species.*

Antibiotic Sensitivity Pattern of *Pseudomonas aeruginosa:*

Total no. of isolates = 88

Antibiotic	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Gentamicin	57	31	64.8%	35.2%
Ciprofloxacin	68	20	77.3%	22.7%
Ceftazidime	52	36	59.1%	40.9%
Amikacin	88	0%	100%	0%
Cefoperazone-Sulbactam	88	0%	100%	0%
Piperacillin-Tazobactam	88	0%	100%	0%

The antibiotic sensitivity pattern of *Pseudomonas aeruginosa* obtained in our study shows that the isolateswere 100% sensitive to Amikacin, Cefoperazone-Sulbactam and Piperacillin-Tazoba-

ctam. 77% of the isolates were sensitive to Ciprofloxacin and 22.7% strains were resistant. 64.8% were sensitive to Gentamicin and 35.2% were resistant. 59.1% were sensitive to Ceftazidime and 40.9% were resistant.

Antibiotic Sensitivity Pattern of *Klebsiella spp*: (28) Isolates

Antibiotic	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Gentamicin	15	13	53.6%	46.4%
Cephalosporin	6	22	21.4%	78.6%
Ceftriaxone	10	18	35.7%	64.3%
Amikacin	24	4	85.7%	14.3%
Ciprofloxacin	10	18	35.7%	64.3%
Piperacillin-Tazobactam	25	3	89.3%	10.7%
Cefoperazone-Sulbactam	28	0	100%	0%

The antibiotic sensitivity pattern of *Klebsiella species* obtained in our study shows that the isolates were 100% sensitive to Cefoperazone-Sulbactam. 89.3% of the isolates were sensitive to Piperacillin-Tazobactam and 10.7% strains were resistant. 85.7% were sensitive to Amikacin and 14.3% were resistant. 53.6% were sensitive to Gentamicin and 46.4% were resistant. 35.7% were sensitive to Ceftriaxone and Ciprofloxacin while 64.3 % were resistant. 21.4% were sensitive to 1stgeneration Cephalosporins and 78.6% were resistant.

Antibiotic Sensitivity Pattern of *E.coli*– (18) Isolates

Antibiotic:	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	0	18	0%	100%
Gentamicin	10	8	55.6%	44.4%
Cephalosporin	3	15	16.7%	83.3%
Ceftriaxone	1	17	5.6%	94.4%
Ciprofloxacin	10	8	55.6%	44.4%
Amikacin	18	0	100%	0%
Cefoperazone-Sulbactam	18	0	100%	0%
Piperacillin-Tazobactam	18	0	100%	0%

The antibiotic sensitivity pattern of *E.coli* species obtained in our study shows that the isolates were 100% sensitive to Cefoperazone-Sulbactam, Amikacin, and Piperacillin-Tazobactam.55.6% were sensitive to Gentamicin and Ciprofloxacin while 44.4 % were resistant. 16.7% of the isolates were sensitive to Istgeneration Cephalosporins and 83.3% strains were resistant. 5.6% were sensitive to Ceftriaxone and 94.4% were resistant. Strains were 100% resistant to Ampicillin.

Antibiotic Sensitivity Pattern of *Proteus vulgaris*- (8) Isolates

Antibiotic:	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	0	8	0%	100%
Gentamicin	0	8	0%	100%
Cephalosporin	0	8	0%	100%
Ceftriaxone	3	5	37.5%	62.5%
Ciprofloxacin	2	6	25%	75%
Amikacin	2	6	25%	75%
Cefoperazone-Sulbactam	7	1	87.5%	12.5%
Piperacillin-Tazobactam	8	0	100%	0%

All the isolates of *Proteus vulgaris* species obtained showed 100% sensitivity to Piperacillin-Tazobactam. Cefoperazone-Sulbactam had sensitivity (87.5%) while 12.5 % were resistant. 37.5% of the isolates were sensitive to Ceftriaxone had sensitivity (37.5%) and resistance (62.5%) respectively. 25% were sensitive to Ciprofloxacin and Amikacin respectively and 75% were resistant to each. Strains were 100% resistant to Ampicillin, Gentamicin and 1stgeneration Cephalosporins.

Antibiotic Sensitivity Pattern of *Proteus mirabilis*– (7) Isolates

Antibiotic:	No. Of Cases		Percentage	
	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	1	6	14.3%	85.7%
Gentamicin	2	5	28.6%	71.4%
Ist generation Cephalosporins	1	6	14.3%	85.7%
Ceftriaxone	3	4	42.9%	57.1%
Ciprofloxacin	3	4	42.9%	57.1%
Amikacin	6	1	85.7%	14.3%
Cefoperazone-Sulbactam	7	0	100%	0%
Piperacillin-Tazobactam	7	0	100%	0%

The antibiotic sensitivity pattern of *Proteus mirabilis* species obtained in our study shows that the isolates were 100% sensitive to Piperacillin-Tazobactam. and Cefoperazone-Sulbactam respectively. 85.7% of the isolates were sensitive to Amikacin and 14.3% strains were resistant. 42.9% were sensitive to Ciprofloxacin and Ceftriaxone respectively and 57.1% were resistant to each. 28.6% were sensitive to Gentamicin and 71.4% were resistant. 14.3% were sensitive to Ampicillin and 1st generation Cephalosporins and 85.7% were resistant.

The antibiotic sensitivity pattern of the only isolate of Enterobacter obtained was sensitive to Cefoperazone-Sulbactam and Piperacillin-Tazobactam and resistant to Gentamicin, Ist generation Cephalosporins, Ceftriaxone, Ciprofloxacin and Amikacin.Outcome: Most patients responded well to treatment, 204(86.8%) recoveredand 31 (13.2%) patients died.

Analysis of Treatment Outcome

Outcome	Number	Percentage
Patients Recovered	204	86.8%
Patients Died	31	13.2%

Out of the 235 cases of Necrotising Fasciitis clinically diagnosed and specimens collected for microbiological investigations, 204 cases (86.8%) were cured after administration of appropriate antibiotic according to the culture and sensitivity pattern. Even after treatment with antibiotics and surgical debridement of the lesion, 31 cases died. The mortality rate was 13.2% in our study.

Discussion

The present study was done to know the spectrum of bacterial aetiological agents of Necrotising Fasciitis with the antimicrobial sensitivity profile of the bacterial isolates. The results were compared and correlated with similar studies conducted by other researchers.

Gender Distribution

From 235 patients who satisfied the clinical criteria for diagnosing Necrotising Fasciitis, the specimens were collected for the study and processed in the Central Microbiology Laboratory at Government Medical College Hospital, Trivandrum. Among these, 203 were males (86.3%) and females only 32 (13.6%) only. There was definite predominance of male population. This is comparable to a study conductedby Dr. Amit Kumar C. Jain et al. in 2008 in Amrita Institute of Medical Sciences and Research Centre at Kochi, India and by N Nischal et al. in 2015 at Bangalore Medical College, Karnataka showing same male predomination in Necrotising Fasciitis .Also the same male predilection pattern was observed in a study conducted by RA Swain et al. at London, UK in 2013. No definite explanation has been given for the sex predominance in Necrotising Fasciitis.

Age Distribution

The upper limit of the age at which Necrotising Fasciitis was observed was 92 years and the minimum age was 19 years .The most common age group in which Necrotising Fasciitis was seen in our study in this institute is between 60 -70yrs (31.48%).This observation is consistent with the finding obtained in the study in Croatia[113] in 2011 and Canada in 2012 .These studies revealed that Necrotising Fasciitis is more common in older age groups (> 50 years of age). It is estimated from the study in Canada[114] that 90–200 cases of Necrotising Fasciitis occur each year in all age groups .Another study conducted in India in 2009 by the department of surgery of Government Medical College and New Civil Hospital at Surat showed that this disease was most common in age group between 25-34 years with male predominance.

Anatomical Site Variation

Necrotising Fasciitis infections occur in different anatomical sites. The most common site of infection observed in our study is the lower limbs 91%. Next common site of infection is perineum (5.5%) followed by upper limbs and abdomen (1.7% respectively). This was consistent with

previous studies conducted by Tang et.al in 2001 where in their case series out of 24 patients,12 cases involved lower limbs. In another study conducted by Wong[92] et.al in 2003 out of 89 patients, 70% were cases with involvement of the lower limbs.Necrotizing infections can occur anywhere in the body, although some anatomic locations are affected more commonly. In a case series[115], these infections were discovered in the extremities (53 percent of cases), perineum or buttocks (20 percent), trunk (18 percent), and head and neck (8.9 percent).

Predisposing Factors

Patients admitted with Necrotising Fasciitis usually have a pre-existing disease which increases their susceptibility to infection. The most commonly associated risk factors in our study were Type 2 DM followed by age >60years, trauma, idiopathic, liver cirrhosis, renal failure, psoriatic skin lesions, peripheral occlusive vascular disease and malignancy.

High blood sugar in patients with Type 2 DM predispose to an environment of low oxygen tension and a rich substrate for bacterial growth. The most common site of infection was lower limb and this can be explained by the fact that most of our patients were diabetics, who are more prone for lower limb infections. This finding is consistent with many other studies.

In a study reported by Sudarsky et al conducted in New York in 1987, Type 2 DM was the most commonly associated risk factor followed by age >60years, trauma, idiopathic, liver cirrhosis, renal failure, and malignancy consistent with the findings of our study.

Clinical Presentation

During the early stage of the disease, the symptoms are non-specific. Physical findings were varied. Tenderness with erythema and edema was the most common finding and was seen in almost all patients. Skin vesicles and bullae, soft tissue crepitus, woody hard texture, hypotension, fever, tachycardia, altered mental status, tachypnoea were the other findings.

In a study conducted by Mathew A et al. at Manipal University, of Karnataka in India in 2010, edema and tenderness were seen in most of the cases compared to erythema (60%) that suggested early signs of the disease and followed by bullae formation noted in 50% of their patients. According to a study by Wong et al in 2003 at Singapore, similar findings served as an important diagnostic clue for Necrotising Fasciitis[92].

Bacteriology

Bacteriology of Necrotising Fasciitis can be polymicrobial involving combinations of anaerobes and facultative anaerobes. As per a study in Washington in 2007, approximately two-thirds of cases were polymicrobial, and one-third were monomicrobial of which majority of cases were gram-positive cocci.

A prospective study from India in 2010 by A. Mathew et al observed the monomicrobial infections (55.6%) to be more common than polymicrobial (44.4%), with the predominant organisms being *Pseudomonas aeruginosa* (23%) followed by *Klebsiella pneumoniae* and *Staphylococcus aureus* (16% each).

The two most common organisms encountered in Hong Kong[116]as per a study done in 2009 were *Streptococci* and *Vibrio species*, the latter being highly virulent and particularly encountered by seafood handlers. One prospective study from Nigeria[117]conducted in the year 2005, reported the commonest offending organisms as *Staphylo-coccus aureus* and *Pseudomonas* in children and adults, respectively.

Positive and Negative Cultures

In our study, culture was positive in 222 cases (94.5%) and culture was negative in 13 cases (5.5%).While analysing the clinical isolates, 94.6% were causing monomicrobial infections and 5.4% were causing polymicrobial infections. Among the clinical isolates, the most common organism isolated and identified was

Pseudomonas aeruginosa (37.44%), next most common was *Staphylococcus aureus* (19.1%), followed by *Klebsiellaspp* (11.91%) then E.coli (7.65%),*Streptococcus pyogenes* (4.25%), *Enterococcus spp*(3.40%) and *Proteus vulgaris* (3.40%), *Proteus mirabilis* (2.97%) and *CoNS* (2.97%), *MRSA* (1.70%), *Enterobacter* (0.42%).

This is consistent with the most recent study published in August 2015 by N Nischal et al. in Karnataka who also had *Pseudomonas aeruginosa* (33%) as the most common isolate followed by *S.aureus* (20%) and *Klebsiella species* (13.3%). Also similar bacterial profile was obtained in a study conducted by Rui Min Foo et al in Singapore published in June, 2015.

Antibiotic Sensitivity Pattern

The treatment of necrotizing fasciitis involves broad-spectrum antibiotics, wide surgical debridement, and hemodynamic support. The choice of antibiotics will vary based on the suspected organisms involved, as well as their local incidence and drug susceptibilities. Most accepted regimens include coverage of gram-positive and gram-negative organisms and anaerobes.[11]

Necrotizing fasciitis due to monomicrobial Gram-negative organisms had been described to have a more fulminant course than Gram-positive organisms[109]. Thirty day mortality was higher in Gram-negative compared with Gram-positive organisms causing Necrotising Fasciitis (42.1% vs. 30.8% respectively) [108].It is conceivable that immunocompromised patients should have a higher mortality than the immunocompetent group. However, in our study of Necrotising Fasciitis patients here in Trivandrum, mortality was relatively low with 86.8% surviving the infection.

This may be attributed to prompt recognition and diagnosis of Necrotising Fasciitis in our centre. All of our patients had received appropriate broad-spectrum antibiotics within 24 hours of clinical presentation and were taken up for surgical debridement when indicated.

Antibiotic sensitivity pattern of *Staphylococcus aureus* isolates showed 100% sensitivity to Cloxacillin, Amikacin, Vancomycin, and 100% resistance to Penicillin. Ist generation Cephalosporins had sensitivity [95.6%] andresistance [4.4%] Gentamicin had sensitivity [93.3%] and resistance [6.7%]. Erythromycin had sensitivity [53.3%] and resistance [46.7%].

Antibiotic sensitivity pattern of *Streptococcus pyogenes* obtained showed that they isolates were 100% sensitive to Penicillin and were 100% resistant to Gentamicin .Ist generation Cephalosporins had sensitivity [80%] and resistance [20%]. Ampicillin had sensitivity [70%] and resistance [30%]. Erythromycin had sensitivity [60%] and resistance [40%].

Enterococcus species isolates obtained were 100% resistant to Penicillin and Gentamicin respectively. Ampicillin had sensitivity [75%] and resistance [25%]. Erythromycin had sensitivity [25%] and resistance [25%].

Methicillin Resistant Staphylococcus aureus (MRSA) isolates obtained were 100% sensitive to Vancomycin. 75% were sensitive to Linezolid, Rifampicin, Amikacin and Clindamycin with 25% resistance to each respectively.

Coagulase Negative Staphylococcus aureus (CoNS) obtained isolates were 100% sensitive to Vancomycin, and 100% resistant to Penicillin and Erythromycin.85.7% of the isolates were sensitive to Amikacin and 14.3% strains were resistant. 57.1% were sensitive to Gentamicin and 42.9 % were resistant. 28.6% were sensitive to 1st generation Cephalosporins and Cloxacillin respectively and 71.4% were resistant to each.

The antibiotic sensitivity pattern of *Pseudomonas aeruginosa* obtained in our study shows that the isolates were 100% sensitive to Amikacin, Cefoperazone-Sulbactam and Piperacillin-Tazobactam. 77% of the isolates were sensitive to Ciprofloxacin and 22.7% strains were resistant. 64.8% were sensitive to Gentamicin and 35.2% were resistant. 59.1% were sensitive to Ceftazidime and 40.9% were resistant.

The antibiotic sensitivity pattern of *Klebsiella species* in our study revealed that the isolates were 100% sensitive to Cefoperazone-Sulbactam. 89.3% of the isolates were sensitive to Piperacillin-Tazobactam and 10.7% strains were resistant. 85.7% were sensitive to Amikacin and 14.3% were resistant. 53.6% were sensitive to Gentamicin and 46.4% were resistant. Both Ceftriaxone and Ciprofloxacin had sensitivity (35.7%) and resistance (64.3%) respectively. 21.4% were sensitive to 1st generation Cephalosporins and 78.6% were resistant.

The E.coli isolates obtained in our study were 100% sensitive to Cefoperazone-Sulbactam, Amikacin, and Piperacillin-Tazobactam, with 100% resistance to Ampicillin. Both Gentamicin and Ciprofloxacin had sensitivity (55.6%) and resistance (44.4%) respectively. Ist generation Cephalosporins had sensitivity (16.7%) and resistance (83.3%). Ceftriaxone had sensitivity (5.6%) and resistance (94.4%).

All the isolates of *Proteus vulgaris* obtained showed 100% sensitivity to Piperacillin-Tazobactam, with 100% resistance to Ampicillin, Gentamicin and Ist generation Cephalosporins. Cefoperazone-Sulbactam had sensitivity (87.5%) and resistance (12.5 %). Ceftriaxone had sensitivity (37.5%) and resistance (62.5%). 25% were sensitive to both Ciprofloxacin and Amikacin with 75% resistance to each.

The antibiotic sensitivity pattern of *Proteus mirabilis* isolates were 100% sensitive to Piperacillin-Tazobactam. andCefoperazone-Sulbactam. Amikacin had 85.7% sensitivityand 14.3% resistance. 42.9% were sensitive to Ciprofloxacin and Ceftriaxone respectively with 57.1% resistance to each. 28.6% were sensitive to Gentamicin and 71.4% were resistant. 14.3% were sensitive to both Ampicillin and Ist generation Cephalosporins with 85.7% resistance respectively.

The antibiotic sensitivity pattern of the only isolate of *Enterobacter* obtained was sensitive to Cefoperazone-Sulbactam and Piperacillin-Tazobactam and resistant to Gentamicin, Ist generation Cephalosporins, Ceftriaxone, Ciprofloxacin and Amikacin.

Conclusion

Bacteriological profile of Necrotising Fasciitis study was conducted in the department of Microbiology, Government Medical College, Thiruvananthapuram during the period of 1 year from May 2014 to April 2015. A total of 235 patients were studied in this regard which included patients of age group above 18 years of both sexes.

- Male preponderance [86.3%] was observed while incidence in females 13.7% only.
- The most common age group affected was between 61 -70yrs (31.48%).
- The common predisposing factors recognised in patients with Necrotising Fasciitis are: Diabetes mellitus [39.57%], age> 60years [31.48%] and trauma [1.76%]
- Lower limbs were the main anatomical site of lesion commonly involved.
- The majority of necrotising fasciitis infections are monomicrobial [94.6%] while polymicrobial was only 5.4%.
- Among the monomicrobial, the most common organism isolated was *Pseudomonas aeruginosa* [37.44%].
- Gram negative organisms account for the majority of bacterial infections [67.5%].
- Gram positive organisms account for 32.5% of infections.
- Among the gram negative bacteria, *Pseudomonas aeruginosa* [58.69%] was the predominant isolate. Other bacteria isolated are *Klebsiella* [11.91%], *E.coli* [7.65%],*Proteus vulgaris* [3.40%], *Proteus mirabilis* [2.97%], and *Enterobacter* [0.42%] species.
- Among gram positive organisms, predominant species were *Staphylococcus aureus* [60.8%] followed by *Streptococcus pyogenes* [4.25%], *Enterococcus* [3.40%], *CoNS* [2.97%], and *MRSA* [1.70%].

- All the isolates of *Pseudomonas aeruginosa* are sensitiveto Amikacin, Piperacillin-Tazobactam and Cefoperazone-Sulbactam whilevarying resistance was observed with Ciprofloxacin (22.7 %), Gentamicin (35.2 %) and Ceftazidime (40.9%).
- All the *Staphylococcus aureus* isolates are resistant to Penicillin but sensitive to Cloxacillin, Amikacin, and Vancomycin. Resistance of varying degree was observed with Erythromycin [46.7%], Gentamicin (6.7%), and 1st generation Cephalosporins (4.4%).
- The mortality rate of the disease in our study was 13.2% only.
- Being a disease associated with significant morbidity and mortality if left untreated, Necrotising fasciitis requires diligent laboratory evaluation with comprehensive antibiotic sensitivity testing. The effective treatment with appropriate antibiotics and the supportive care given such as wound debridement with proper cleaning of the infected site with antiseptics has significantly reduced the morbidity and mortality rate of Necrotising fasciitis. After receiving our microbiological culture report, the surgeons promptly managed accordingly and most of the patients thus recovered.
- Proper collection of sample at the proper time enabled us to isolate the bacteria in pure culture and the proper treatment could be started in the early stage of the disease. The co-ordination between the microbiologist and clinician made the management of Necrotising fasciitis effective in reducing the mortality and morbidity of this disease in our institution.

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