

Research Article

PREVALENCE OF AEROBIC BACTERIA CAUSING WOUND INFECTION IN PATIENTS WITH FILARIAL ELEPHANTIASIS IN A TERTIARY CARE HOSPITAL OF KOLKATA, INDIA

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ABSTRACT: Lymphatic filariasis, thought to be a neglected tropical disease (NTD) globally, is caused by microscopic, thread-like nematodes. The present study was conducted to identify the microorganisms in the ulcerative wounds of filarial elephantiasis. A total of 100 samples were collected and studied from the patients attended on Filaria OPD, School of Tropical medicine, Kolkata, India. *Staphylococcus aureus* (46.67%) was identified as the predominant organism among the different aerobic bacteria present in the ulcer, followed by *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella sp.*, *Enterococcus sp.*, *Escherichia coli* and *Enterobacter sp.*

Key words: Filarial elephantiasis, Wound infection, Microorganisms.

INTRODUCTION

Lymphatic filariasis is a vector borne (mosquitoes) neglected tropical disease due to infection by filarial worms (e.g., *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori*). In India, *Wuchereria bancrofti* is predominant species contribution to approximately 98.4% of the problem in the country. Adult filarial worms invade the lymphatics of human causing lymphatic obstruction and the pathological changes leading to various clinical manifestations (Babu and Nutman 2005, NVBDCP 2013, Park 2015).

Globally, around 120 million people in 83 countries are affected by this disease, which is ranked as second most common cause of physical disability (WHO Report 1995). According to WHO, India, Indonesia, Nigeria, and Bangladesh alone contribute about 70% of the infection worldwide (<https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis>, NVBDCP 2013).

Lymphatic filariasis is a leading cause of permanent

disability worldwide. Communities frequently shun and reject women and men disfigured by the disease. Affected people often cannot work because of their disability, which harms their families and communities. People can suffer from Lymphoedema and elephantiasis, and in men, swelling of the scrotum is called a hydrocele (<https://www.cdc.gov/parasites/lymphaticfilariasis/index.html>). Lymphoedema is a progressive condition that can have a marked physical and psychological impact on affected patients and significantly reduce the quality of life. The ulcers on chronic lymphoedema patients, which often makes it impossible for them to work. If left untreated, tends to progress or worsen. Ulcers in lymphoedema patients, therefore, represent not only a medical but also a psychological problem (Karnasula 2012, Fu *et al.* 2012).

As a result of lymphatic obstruction, protein rich lymph fluid accommodates in the subcutaneous tissue leading to lymphoedema. When the infection is associated with recurrent attacks of adeno-lymphangitis, lymphatic

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obstruction gradually progress more and resulting permanent accumulation of lymph fluid in tissue, which initiates hyperplasia and hypertrophy of subcutaneous tissue, overlying skin undergoes hyperkeratosis, papillomatous proliferation and nodular warty growth and chronic non healing ulcers (Babu and Nutman 2005, Burri *et al.* 1996). This newly form tissue is relatively avascular and susceptible to various bacterial infections. Due to relative avascularity, it is very difficult to control of the infection. Moreover, since these bacteria are resistant to conventional antibiotics.

The aim of the present study was

1. Isolation and identification of aerobic bacteria present in the ulcerative wound, and
2. Antibiotic sensitivity pattern of the isolated bacteria.

MATERIALS AND METHODS

The present study aimed to isolate and identify aerobic bacteria wounds in patients with filarial elephantiasis and determine their drug sensitivity pattern to prevent further damage to subcutaneous tissues and lymphatics of the affected part.

Source of sample

Our study was conducted on wound samples of patients with filarial elephantiasis with ulcerations, attended the Filaria OPD, School of Tropical Medicine, Kolkata, India. The samples received were from different districts of West Bengal as well as from outside of West Bengal. A total of 100 wound samples were collected from the patients.



Fig. 1. Elephantiasis with ulcer.

Table 1. Distribution of different species of bacteria among the total isolated aerobic cultures.

Organisms isolated	No. of strain	Percentage
<i>Staphylococcus aureus</i>	35	46.67%
<i>Pseudomonas aeruginosa</i>	13	17.33%
<i>Proteus mirabilis</i>	11	14.67%
<i>Klebsiella pneumoniae</i>	5	6.67%
<i>Klebsiella oxytoca</i>	2	2.66%
<i>Enterococcus sp</i>	5	6.67%
<i>Escherichia coli</i>	3	4%
<i>Enterobacter sp.</i>	1	1.33%
Total	75	100%

Identification of the organisms

After cleaning the wound area with normal saline (NSS), two swabs were taken from the deeper portion of the wound. One swab was used for direct microscopical examination after gram staining to see pus cells and characteristics of organisms if present. Another swab was streaked on MacConkey's agar and blood agar plate and incubated aerobically at 37°C on bacteriological incubator for up to 48 hours. If there was growth on agar plate, colonies were evaluated to see the colony characters, colour of the colony or haemolysis on blood agar. Then microscopical examination of the colonies was done after gram staining to see the morphology and characteristics of the organisms. Then definitive identification of the organism up to species level were done by different biochemical reactions (Catalase test, Coagulase test, Bile esculin test, Indole test, Triple sugar Iron agar test, MR and VP test, Citrate utilization test, Urea hydrolysis test,

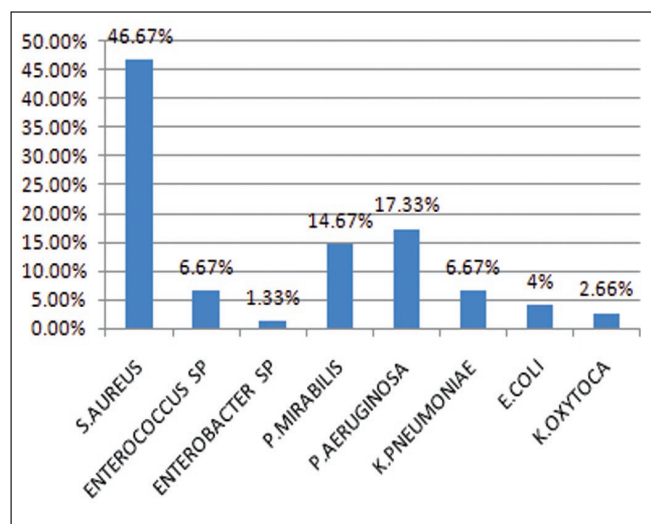


Fig. 2. Diagram showing distribution of bacteria among isolated aerobic cultures.

Table 2. Drug sensitivity/resistance pattern (%) of different isolated gram negative bacteria (Organism vs antimicrobials).

Antibiotic	Result	Sensitivity pattern of bacterial isolate in %				
		<i>P. aeruginosa</i>	<i>P. mirabilis</i>	<i>Klebsiella Sp.</i>	<i>E. coli</i>	<i>Enterobacter Sp.</i>
Amikacin	S	76.92	72.73	57.14	100	100
	R	23.08	27.27	42.86	0	0
Amoxy clav.	S	0	0	28.57	33.33	0
	R	100	100	71.43	66.67	100
Cefixime	S	0	0	42.86	66.67	0
	R	100	100	57.14	33.33	100
Ceftazidime	S	69.23	-	-	-	-
	R	30.77	-	-	-	-
Ciprofloxacin	S	46.15	100	57.14	66.67	0
	R	53.85	0	42.86	33.33	100
Co-trimoxazole	S	0	27.27	28.57	0	0
	R	100	72.73	71.43	100	100
Gentamicin	S	61.53	81.82	57.14	100	100
	R	38.47	18.18	42.86	0	0
Imipenem	S	92.31	81.82	42.86	100	100
	R	7.69	18.18	57.14	0	0
Levofloxacin	S	0	36.36	71.43	66.67	0
	R	100	63.64	28.57	33.33	100
Meropenem	S	76.92	81.82	85.71	100	100
	R	23.08	18.18	14.29	0	0
Ofloxacin	S	0	54.55	42.86	33.33	0
	R	100	45.45	57.14	66.67	100
Piperacillin+ Tazobactam	S	92.31	100	71.43	100	100
	R	7.69	0	28.57	0	0
Ceftriaxone	S	23.08	72.73	57.14	100	100
	R	76.92	27.27	42.86	0	0

Different sugar media and different decarboxylation test, etc.) (Mackie and McCartney 1996, Koneman 2005, Bailey and Scott 2013).

Brief description of the procedures followed

Swabs from infected wounds were taken from ulcers of one hundred (100) patients with filarial elephantiasis and aerobic culture was done as per routinely done on Microbiology laboratory.

Wound samples were cultured aerobically by using

routine culture media (MacConkey's agar and Blood agar) and incubated at 37°C for 48 hours. After identification of organisms antibiotic sensitivity of isolated aerobic bacteria was done by Kirby-Buurs disk diffusion method by using Muller-Hinton agar and antibiotic were selected as per CLSI guideline (CLSI 2016).

RESULTS AND DISCUSSION

A total of 100 swabs were examined. Among the 100 samples, 75 samples showed aerobic bacterial growth.

Table 3. Drug sensitivity/resistance pattern (%) of different isolated gram positive bacteria (Antimicrobials vs organisms).

Antibiotic	Result	Sensitivity pattern of bacterial isolates in %	
		<i>S. aureus</i>	<i>Enterococcus sp</i>
Amoxy-Clav	S	57.16	-
	R	42.84	-
Azithromycin	S	62.85	-
	R	37.15	-
Cefixime	S	28.57	-
	R	71.43	-
Ceftriaxone	S	60	-
	R	40	-
Ciprofloxain	S	42.86	20
	R	57.14	80
Co-Trimoxazole	S	31.42	-
	R	68.58	-
Levofloxacin	S	51.43	60
	R	48.57	40
Linezolid	S	94.29	100
	R	5.71	0
High Level Gentamicin	S	-	60
	R	-	40
Piperaciillin+ Tazobactum	S	91.43	-
	R	8.57	-
Vancomycin	S	82.86	80
	R	17.14	20

The presence of different species of organisms in the infected wounds is shown in Table 1 and Fig 2. Different aspects of the sensitivity of the organisms are shown in Tables 2-4.

It is hypothesized that lymph stasis in limbs of Indian patients with filarial lymphoedema and exposure to a highly contaminated environment predispose to bacterial colonization of skin and penetration of microbes to deeper tissues and the lymphatics of the extremities (Fig. 1). Moreover, the colonizing bacteria may, under certain conditions (skin microtrauma, insect bites), becomes activated and find their way from tissues and fluids of the limbs to the blood circulation (Olszewski *et al.* 1999).

In a total of 100 patients with filarial elephantiasis, only 75 patients showed growth of aerobic bacteria. Among them, *Staphylococcus aureus* is the predominant organism (46.67%), followed by *Pseudomonas*

Table 4. Percentage of sensitivity to all antibiotic among five isolated gram negative bacteria.

Antibiotics	Sensitivity
Amikacin	81.34
Amoxy-clav	12.38
Cefixime	21.91
Ceftazidime	69.23
Ciprofloxain	53.99
Co-trimoxazole	11.17
Ceftriaxone	70.59
Gentamicin	80.1
Imipenem	83.34
Levofloxain	34.89
Meropenem	88.89
Ofloxacin	26.15
Piperacillin-Tazobactum	92.75

aeruginosa (17.33%), *Proteus mirabilis* (14.67%), *Klebsiella sp* (9.33%), *Enterococcus sp* (6.67%), *Escherichia coli* (4%) and *Enterobacter sp* (1.33%). As per the study by Rose Cooper, though *Staphylococcus aureus* is the common organism along with *Streptococcus pyogenes* in cellulitis in lymphoedema patients, gram-negative bacilli can be implicated (Cooper and White 2009).

In our study, *Staphylococcus aureus* is the predominant isolated organisms (46.67%) followed by *Pseudomonas aeruginosa* (17.33%). Several studies from chronic leg ulcers without filarial elephantiasis showed that most common responsible bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Mihai *et al.* 2014, Bowler and Davies 1999, Lim *et al.* 2006).

Also, as per Pal *et al.* (2015) from costal area of Odisha, bacterial pathogens with filarial encephalitis patients, *Staphylococcus aureus* was the predominant bacteria (55.1%). In our study, prevalence of *S. aureus* is slightly lower (41.67%).

Though *S. aureus* is the predominant isolated organisms in our study but they are more resistant to commonly used oral antibiotic like Amoxy-clav, Cefixime, Levofloxacin than parenteral antibiotics like Piperacillin-tazoctum, Ceftriaxone. It also seen that though Linezolid and Vancomycin are highly sensitive against gram positive cocci, but in our study some amount of resistance found against them.

Among the isolated gram negative bacilli, *Pseudomonas aeruginosa* is the predominant organisms. *Pseudomonas sp.* can be commonly found on the skin and healthy people do not normally develop *Pseudomonas*

infection. It is considered to be opportunistic and most frequently cause disease who are immune compromised like steroid therapy, diabetes etc. (Bush 2020, Diggle and Whiteley 2020, Wu 2021). Several studies showed that *Pseudomonas aeruginosa* is a common pathogen causing wound infection along with *Staphylococcus aureus* specially in diabetes patients (Shankar *et al.* 2005, Abdulrazak *et al.* 2005).

It is also seen that like gram positive organisms, gram negative bacilli are mostly sensitive to parenteral antibiotics like Piperacillin-Tazobactam, Meropenem, Amikacin, Gentamicin and more resistant to commonly used oral antibiotics like Levofloxacin, Amoxy-clav, Cefixime. Similar pattern of bacterial infections of wounds by different Multi-Drug Resistance organisms are reported (Abedin *et al.* 2022).

CONCLUSION

The result of our study demonstrate the prevalence of secondary aerobic bacterial infection in patients with filarial elephantiasis and their antibiogram profile for effective antibiotic selection to prevent further disease progression or damage of affected area.

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