



## Bacteria associated with ocular infections of out-patients visiting optical clinic in Owerri, Imo State, Nigeria

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Open Access Research Journal of Science and Technology, 2022, 04(01), 019–030

Publication history: Received on 14 September 2021; revised on 24 October 2021; accepted on 26 October 2021

Article DOI: <https://doi.org/10.53022/oarjst.2022.4.1.0056>

### Abstract

The study was done to determine the prevalence and types of bacteria associated with ocular infections from out-patients attending optical clinic in Owerri, Imo State at the time of study. Eye swab specimens were collected aseptically from the conjunctival sac of each patient using sterile swab sticks. A total of fifty specimens were collected from patients comprising fourteen (14) males and thirty-six (36) females with ocular infections. Standard microbiological and biochemical investigations were carried out on the samples to isolate and identify the organisms involved. Results revealed that all specimens had bacterial growth. A total of fifty seven (57) bacterial isolates were obtained out of which 35 (61.4%) were Gram positive and 22 (38.6%) Gram negative. Mixed bacterial infections were observed in some specimen. The isolates fell into eleven species with varying prevalence as follows; *Bacillus subtilis* was the predominant species, 17 (29.8%) recovered followed by *Corynebacterium* sp. and *Pseudomonas aeruginosa* with the same prevalence of 8 (14.0%). *Haemophilus* sp. had a prevalence of 7 (12.3%), *Staphylococcus aureus*, 5 (8.8%) and *Lactobacillus* sp. 3 (5.3%). *Klebsiella* sp., *Citrobacter* sp. and *Proteus* sp. had same prevalence 2 (3.5%) while *Streptococcus*, *Listeria* and *Neisseria* spp. were the least isolated with 1 (1.8%) respectively. The mean viable count/prevalence rate was higher with the female gender while the age group 31 - 40 years had highest mean viable count. This implies that broad spectrum bacterial species are associated with ocular infections in Imo State at varying prevalence rates which are seemingly high. There is therefore need for proper diagnosis of eyes infections to ascertain the type and abundance of bacteria involved before administration of treatment regimen.

**Keywords:** Prevalence; Bacteria; Ocular Infections; Diagnosis; Imo State

### 1. Introduction

Ocular infections are eye conditions, diseases and vision problems; frequently reported clinical manifestations include conjunctivitis, keratitis, blepharitis, hordeolum and dacryocystitis [1]. Ocular infections if left untreated can damage the structures of the eye leading to visual impairments and blindness [2]. Even though the eye is hard and protected by the continuous flow of tears which contain antibacterial compounds, inflammation and scarring once occurred may not be easily resolved and requires immediate management [3].

Bacteria have been reported as the major causative agents of ocular infections worldwide [4, 5]. They gain access into the eye through different routes and cause infections [6]. Trauma, surgery and systemic diseases are among the contributory risk factors to ocular infections by bacteria [7].

Previous studies in different countries have reported the prevalence of bacterial isolates among patients with ocular infections. Amsalu *et al.* [8] reported a prevalence of 143 (50.9%) bacterial isolates with Gram positive cocci as the most common isolates. *Staphylococcus aureus* 30 (21%) was the predominant species followed by coagulase-negative

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*Staphylococci* (CoNS) 26 (18.2%) and *Streptococcus pneumoniae* 20 (14.0%). In another study by Tewelde *et al.* [9], a high prevalence of 148 (74.7%) was reported with *S. aureus* 42 (28.4%) as the most frequent bacteria followed by *P. aeruginosa* 31 (21%) and *S. pneumoniae* 20 (13.5%). A similar study by Shahaby *et al.* [10] reported a prevalence of 70 (78.7%) with *S. aureus* 54 (19.6%) as the predominant bacteria followed by coagulase-negative *Staphylococci* (CoNS) 45 (16.4%) and *B. subtilis* 42 (15.3%) while a higher prevalence of 168 (88%) was reported by Getahun *et al.* [11] with *Staphylococcus aureus* 96 (50.3%) as the predominant bacteria followed by coagulase-negative *Staphylococci* (CoNS) 64 (33.5%) and *Klebsiella* species 9 (4.7%).

These bacteria have varying debilitating activities on the eye ranging from mild to fatal effects. Infections by single species of bacteria may be less difficult to treat than mixed infections. To mitigate the burden of ocular infections, physicians need to comply with etiologic approach of diagnosis and treatment regimen. Global reports show that the prevalence and types of bacteria may not be exactly the same in every part of the world [2]. There has been no published data on prevalence and types of bacteria associated with ocular infections in Imo State to guide empirical diagnosis and treatment.

This study was therefore done to determine the prevalence and types of bacteria associated with ocular infections in Imo State, Nigeria with a view to providing baseline data on bacterial infections of the eye across different age groups and between gender which will be useful for medical practitioners as well as the unassuming populace in Imo state. This is expected to aid empirical treatment of ocular infections.

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## 2. Methodology

To achieve the set objective of the study, the following standard protocols were used.

### 2.1. Location of Study and study subjects

This study was carried out in Optical clinics in Imo State. A total of fifty (50) Out-patients having different types of eye infections attending the clinic within the period of the study and consenting to the study were recruited as study subjects.

### 2.2. Ethical permission and informed consent

Ethical permission was sought and obtained from the ethical committee of School of Health Technology, Federal University of Technology, Owerri.

The research subjects' informed consent was obtained prior to recruitment for the study.

### 2.3. Sample collection and preparation

A total of fifty (50) specimens were aseptically collected from the conjunctival sac, of respective subjects using sterile swab sticks. The swab sticks with specimen were labeled appropriately and taken to the laboratory within 30 mins of collection for investigations.

Interviewee and interviewer administered questionnaires were used to collect demographic data and clinical history of the subjects.

### 2.4. Isolation and Characterization of organisms

The specimens were inoculated into peptone broth in test tubes and maintained overnight. Then, 1 ml of the overnight peptone broth culture was transferred into appropriately labeled sterile petridishes containing sterile solid culture media (nutrient, blood and MacConkey agar) respectively in duplicates. The inoculum was evenly spread over the surface of the solid media according to standard methods [12, 13]. The respective plates were incubated for 24 hrs at 37°C. the plates were then observed for microbial growth/colonies. The observations were appropriately recorded accordingly. Mean colony counts of duplicate plates were recorded.

### 2.5. Characterization/Identification of Isolates

Gram staining and other standard biochemical tests; Indole, citrate utilization, oxidase, coagulase, motility, hemolysin, sugar fermentation and catalase tests were carried out to appropriate characterize the isolates using the protocols [12, 13].

## 2.6. Statistical analysis

Frequencies and percentages were calculated for study variables. Other statistical tools used were ANOVA and T- test to make inferences from data.

## 3. Results

Out of these fifty (50) subjects recruited, 14 (28%) were males and 36 (72%) females. These were all adults from age 16 and were categorized under 5 distinct age groups. The highest percentage (38) of subjects were within the age group 21-30 years while the least percentage (6) were of age group 41-50 years (Table 1).

Culture results revealed presence of fifty seven (57) bacterial isolates from the 50 specimen cultured. The Gram reaction distinguished the isolates to be Gram positive 35 (61.4%) and Gram negative 22 (38.6%) bacteria (Tables 2 and 3). These fell into twelve (12) distinct species (Tables 2 and 3). The predominant bacterial species was *Bacillus* sp. 17 (29.8%) while *Streptococcus* sp., *Listeria* sp., and *Neisseria* sp. were the least with 1 (1.8%) each (Table 3).

The relationship between age, sex and bacterial load in ocular infections are shown in (Table 4).

The prevalence rate of bacteria was higher among the female gender within the age group 21 -30 years while the least occurrence was among the females within age group 41-50 years. The total plate counts ranged from  $1.0 \times 10^2$  cfu / ml to  $2.8 \times 10^2$  cfu / ml (Table 4). Differences in bacterial isolation between both sexes (P- value > 0.05) and across various age groups (P-value > 0.05) were not statistically significant.

**Table 1** Categories of study subjects according to age group and gender

Gender	Age groups (years)					Total Number (%)
	> 20	21 -30	31 - 40	41 - 50	Above 50	
Male	1	8	3	1	1	14 (28)
female	7	11	7	2	9	36 (72)
Total number (%)	8 (16)	19 (38)	10 (20)	3 (6)	10 (20)	50 (100)

**Table 2** Characterization/Identification of Bacterial Isolates

Isolates ID	Cultural characteristics	Morphological characteristics	Gram stain	Motility	Coagulase	Catalase	Indole	Oxidase	Citrate	L	S	G	Probable organism
1	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	$\beta$ <i>Bacillus subtilis</i>
2	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	$\beta$ <i>Bacillus subtilis</i>
3	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	$\beta$ <i>Bacillus subtilis</i>
4	Raised translucent, grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	$\alpha$ <i>Corynebacterium</i> sp.
5	Large, flat spreading colonies on blood agar with greenish color on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	$\beta$ <i>Pseudomonas aeruginosa</i>
6	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	$\beta$ <i>Bacillus</i> sp.
7	Translucent, convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacilli	-	-	-	+	+	+	-	-	+	+	$\gamma$ <i>Haemophilus</i> sp.
8	Raised translucent, grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	$\alpha$ <i>Corynebacterium</i> sp.
9A	Large, flat spreading colonies on blood agar with greenish color on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	$\beta$ <i>Pseudomonas aeruginosa</i>

9B	Round, smooth, grey and convex with a clearly defined edge on blood agar.	Gram negative cocci in pairs	-	-	-	+	-	+	+	-	+	+	β	<i>Neisseria</i> sp.
10	Large, flat spreading colonies on blood agar with greenish color on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	β	<i>Pseudomonas aeruginosa</i>
11	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus</i> sp.
12	Raised, translucent grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium</i> sp.
13	Muroid colonies, yellowish on blood agar.	Gram positive cocci in clusters	+	-	+	+	-	-	+	+	+	+	β	<i>Staphylococcus aureus</i>
14	Muroid colonies, yellowish on blood agar.	Gram positive cocci in clusters	+	-	+	+	-	-	+	+	+	+	β	<i>Staphylococcus aureus</i>
15A	Large, flat spreading colonies on blood agar with greenish color on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	β	<i>Pseudomonas aeruginosa</i>
15B	Large flat colonies on nutrient agar	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus</i> sp.
16	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus</i> sp.
17	Large, grey white muroid colonies on blood agar.	Gram negative rods	-	-	-	+	-	-	+	+	+	+	α	<i>Klebsiella</i> sp.

18	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
19	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
20	Large, grey white mucoid colonies on blood agar.	Gram negative rods	-	-	-	+	-	-	+	+	+	+	α	<i>Klebsiella sp.</i>
21	Red pinkish colonies on MacConkey agar.	Gram negative rods	-	+	-	+	-	-	+	-	+	+	γ	<i>Proteus sp.</i>
22A	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
22B	Translucent convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacillus	-	-	-	+	+	+	-	-	+	+	γ	<i>Haemophilus sp.</i>
22C	Large, colonies greenish color on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	β	<i>Pseudomonas aeruginosa</i>
23A	Pale to pink colored colonies on MacConkey agar.	Gram negative rods	-	+	-	+	-	-	+	+	+	+	γ	<i>Citrobacter sp.</i>
23B	Raised translucent, grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium sp.</i>
24	Red pinkish colonies on MacConkey agar.	Gram negative rods	-	+	-	+	+	-	-	-	-	+	α	<i>Proteus sp.</i>
	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
25A	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>

25B	Translucent convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacillus	-	-	-	+	+	+	-	-	+	+	γ	<i>Haemophilus sp.</i>
26	Large, colonies greenish color on nutrient agar	Gram negative rods	-	+	+	-	-	+	+	-	+	+	β	<i>Pseudomonas aeruginosa</i>
27	Translucent convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacillus	-	-	+	+	+	+	+	-	+	+	γ	<i>Haemophilus sp.</i>
28	Translucent, convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacillus	-	-	-	+	+	+	+	-	+	+	γ	<i>Haemophilus sp.</i>
29	Mucoid colonies yellowish on blood agar.	Gram positive cocci in clusters	+	-	+	+	-	-	+	+	+	+	β	<i>Staphylococcus aureus</i>
30	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
31	Raised, translucent grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium sp.</i>
32	Pale to pink colored colonies on macConkey agar.	Gram negative rods	-	+	-	+	+	-	+	+	+	+	γ	<i>Citrobacter sp.</i>
33	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i>
34	Small, smooth and whitish-grey colonies on blood agar.	Short Gram positive rods	+	+	-	+	-	-	+	+	-	+	β	<i>Listeria sp.</i>

35	Small to medium grey colonies on blood agar.	Gram positive rods	+	-	-	-	-	-	-	+	+	+	α	<i>Lactobacillus</i> sp.
36	Raised, translucent grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium</i> sp.
37	Yellow cream colonies on blood agar.	Gram positive cocci in clusters	+	-	-	+	-	-	-	+	+	+	γ	<i>Staphylococcus aureus</i>
38	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus subtilis</i> .
39	Large, colonies greenish colour on nutrient agar	Gram negative rods	-	+	-	+	-	+	+	-	-	-	β	<i>Pseudomonas aeruginosa</i>
										L	S	G		
40	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus</i> sp.
41	Gray mucoid colonies on blood agar.	Gram positive cocci in chains	+	-	+	-	-	-	-	-	+	+	β	<i>Streptococcus pyogenes</i>
42	Raised, translucent grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium</i> sp.
43	Translucent, convex or flat pinpoint colonies in blood agar.	Gram negative short rods or coccobacillus	-	-	+	+	+	+	+	-	+	+	γ	<i>Haemophilus</i> sp.
44	Small to medium grey colonies on blood agar	Gram positive rods	+	-	-	-	-	-	-	+	+	+	α	<i>Lactobacillus</i> sp.
45	Small to medium grey colonies on blood agar.	Gram positive rods	+	-	-	-	-	-	-	+	+	+	α	<i>Lactobacillus</i> sp.



46	Translucent, convex or flat pinpoint colonies on blood agar.	Gram negative short rods or coccobacillus	-	-	+	+	+	+	+	-	+	+	γ	<i>Haemophilus sp.</i>
47	Raised, translucent grey colonies on blood agar.	Gram positive rods	+	-	-	+	-	-	+	-	-	+	α	<i>Corynebacterium sp.</i>
48	Yellow cream colonies on blood agar.	Gram positive cocci in clusters	+	-	-	+	-	-	-	+	+	+	γ	<i>Staphylococcus aureus</i>
49	Large, flat spreading colonies on blood agar.	Gram negative rods	-	+	-	+	-	+	+	-	-	-	β	<i>Pseudomonas aeruginosa</i>
50	Large, flat colonies on nutrient agar.	Gram positive rods with spores	+	+	-	+	-	-	+	-	+	+	β	<i>Bacillus sp.</i>

KEY: + = Positive; - = Negative; L = Lactose; S = Sucrose; G = Glucose; α = alpha; β = Beta; γ = Gamma

**Table 3** Frequency of Bacterial isolates from patients with Ocular Infections

Bacterial Isolates	Number Isolated	Frequency (%)
<b>Gram positive</b>		
<i>Bacillus</i> sp.	17	29.8
<i>Corynebacterium</i> sp.	8	14.0
<i>Staphylococcus aureus</i>	5	8.8
<i>Lactobacillus</i> sp.	3	5.3
<i>Streptococcus pyogenes</i>	1	1.8
<i>Listeria</i> sp.	1	1.8
Sub Total	35	61.4
<b>Gram negative</b>		
<i>Pseudomonas aeruginosa</i>	8	14.0
<i>Haemophilus</i> sp.	7	12.3
<i>Klebsiella</i> sp.	2	3.5
<i>Citrobacter</i> sp.	2	3.5
<i>Proteus mirabilis</i>	2	3.5
<i>Neisseria</i> sp.	1	1.8
Sub Total	22	38.6
Grand Total	57	100

**Table 4** Viable bacterial count according to age group and gender

Viable bacterial count (cfu/ml) across gender	Viable bacterial count (cfu/ml) across Age groups (years)					Total
	10 - 20	21 - 30	31 - 40	41 - 50	Above 50	
Male	0	$4.9 \times 10^2$	$6.0 \times 10^2$	0	$3.0 \times 10^2$	$1.39 \times 10^3$
female	$1.19 \times 10^3$	$1.8 \times 10^3$	$1.6 \times 10^3$	$2.1 \times 10^2$	$1.71 \times 10^3$	$8.4 \times 10^3$
Total viable count	$1.19 \times 10^3$	$2.29 \times 10^3$	$2.2 \times 10^3$	$2.1 \times 10^2$	$2.01 \times 10^3$	$9.79 \times 10^3$
Mean viable count	$1.49 \times 10^2$	$1.21 \times 10^2$	$2.2 \times 10^2$	$7.0 \times 10^1$	$2.01 \times 10^2$	$1.96 \times 10^2$

#### 4. Conclusion

Results revealed that all the 50 specimen collected had bacterial growth with seven having mixed microbial growth (Table 2), this implies that bacteria are associated with most eye infections. Earlier studies have also implicated various bacteria species in different ocular infections [8, 10, 11] some of which were also isolated in this study with varying prevalence rates. Bacteria have also been known to be versatile and ubiquitous [13, 14]. One of the isolates in this study *Pseudomonas aeruginosa* is also a known recalcitrant organism [12, 14].

Results also revealed that broad spectrum of bacteria are associated with eye infections in Imo state with Gram positives being more prevalent (61.4%) than Gram negative bacteria (38.6%) (Table 3). This corroborates the report of Amsalu *et al.* [8] of a prevalence of 61.5% for Gram positive bacteria and 38.5% for Gram negative bacteria in their study.

Getahun *et al.* [11] reported a much higher prevalence of 88% for Gram positive bacteria and lower prevalence of 23 (12%) for Gram negative bacteria. The disparity may be due to the kinds of infections studied.

The predominant bacterial isolates were *Bacillus* sp. 17 (29.8%) followed by *Corynebacterium* sp. 8 (14.0%) and *Pseudomonas* sp. 8 (14.0%) (Tables 2 and 3). This finding does not agree with previous studies where *Staphylococcus* sp. had been reported as being the predominant isolate [8, 9, 10, 15, 16]. A study by Iwalokun *et al.* [17] showed that Gram positive *bacilli* are known to cause 22.6% of conjunctivitis cases where *Corynebacterium* sp. 25 (16.1%) was the predominant Gram positive *bacilli* followed by *Bacillus* sp. 10 (6.5%). However, Tewelde *et al.* [2] reported that predominant pathogens may not be exactly the same in all areas of the world. This has also been revealed by the current study though the type of pathogens isolated from Imo State in this current study showed similarities with those isolated in other parts during earlier studies [8, 11, 17]

The mean viable count revealed age group 31 - 40 having the highest count ( $2.2 \times 10^2$  cfu/ml) followed by age group above 50 with count of  $2.01 \times 10^2$  cfu/ml with age group 41 – 50 years having the least prevalence (Table 4). However, the difference in prevalence rates across the age groups was not statistically significant. Age therefore did not seem to affect the prevalence of bacteria in ocular infections.

Females were observed to be more infected than their male counterpart in this study (Table 4). This corroborates the study by Lewallen *et al.* [18] who reported that feminine gender was significant risk factor for some eye diseases. However, the prevalence was not found to be statistically associated with gender in this study.

Conclusively, this study has revealed baseline data of types and prevalence of bacteria in ocular infections. Knowledge of the prevalence and types of bacteria associated with ocular infections helps for prompt diagnosis and commencement of appropriate treatment, proper management of such infections by ensuring patients and health care workers constantly wash their hands to avoid cross- infection, limitation of hospital- acquired infection by healthcare workers. It is also expected to arm health educators for proper development and teaching of appropriate infection control policy with regular reinforcement and review in Imo State. These are expected to help reduce/curb the menace of bacterial infections of the eye across the State.

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## Compliance with ethical standards

### *Acknowledgments*

We acknowledge all authors whose works were cited in this work.

### *Disclosure of conflict of interest*

The authors hereby declare that there is no conflict of interest among them or with any person/organization

### *Statement of ethical approval*

Ethical permission was sought and obtained from the ethical committee of School of Health Technology, Federal University of Technology, Owerri.

### *Statement of informed consent*

The research subjects' informed consent was obtained prior to recruitment for the study.

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