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Prevalence and antibiogram profile of bacteria associated with throat infections in Akure metropolis, Ondo State, Nigeria

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ABSTRACT

Background: Throat infections are one of the commonest causes for visits to health care physicians. It was hypothesized that some rare Gram negative bacteria could be implicated in respiratory infections. The molecular dynamics of clinically-significant Gram negative bacteria associated with lower respiratory tract infections was also ascertained. This study investigated the prevalence and antibiotic susceptibility pattern of bacteria associated with throat infections in a hospital setting. **Methods:** Two hundred and five (205) throat swabs were collected from patients at the study area. Bacterial isolates were identified based on their morphological and biochemical profile. The molecular identity of selected multidrug resistant bacterial isolates was conducted via DNA extraction, polymerase chain reaction (PCR) amplification, sequencing and genome blasting. Antibiotic susceptibility of the test bacterial isolates was evaluated using Kirby-Bauer disc diffusion method. **Results:** The highest frequency of carriers of throat pathogens was obtained among male patients between ages 31 and 40 years. *Streptococcus pneumoniae* (28%) had the highest prevalence while *Escherichia coli* (4%) had the least. One of the enumerated *Enterobacter* species from the samples was further identified via 16S ribosomal ribose-nucleic acid (rRNA) as *Enterobacter bugandensis* MH712497.1. *Staphylococcus aureus* and *Streptococcus pyogenes* were resistant to gentamicin whilst both Gram-negative bacteria were susceptible to ciprofloxacin at 21.00±0.58 mm and 20.00±0.58 mm respectively. **Conclusion:** This study deduced that *Streptococcus* species is most implicated bacteria responsible for throat infections in clinical setting. Molecularly-identified *Enterobacter bugandensis* from throat infections in this study has recently being implicated as a fresh enterobacteria associated with severe clinically-significant infection.

Introduction

Throat infections are the commonest cause for visits to health care physicians [1]. They may be associated with mild to severe pains, fever, headache [2] running or stuffy nose and fullness of the ear [3]. Severe respiratory tract infections (SRIs) are comprised of upper respiratory tract infections (URIs) and lower respiratory tract infections (LRIs). Upper respiratory tract infections are associated with infections at or above the larynx commonly provoked by viruses and include rhinitis, sinusitis, ear infections, acute pharyngitis, epiglottitis and laryngitis. Lower respiratory tract infections are comprised of bacterial or viral maladies taking place

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beneath the larynx and include bronchitis, bronchiolitis and pneumonia [4]. Most of the URIs are caused by viruses but secondary infections occur after the viral infections by bacteria. According to [5], respiratory infections are the second cause of death in children due to pneumonia in developing countries. More than 225 pathogens are responsible for URIs, the most common bacteria involved are *Streptococcus pyogenes*, *S. pneumoniae, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter koseri* and *Acinetobacter baumanii* [1, 6].

It was hypothesized that some rare Gram negative bacteria could be implicated in respiratory infections. *Enterobacter* infections can include bacteremia and LRTIs [7]. They are also of clinical importance as nosocomial opportunistic pathogens and species of the genus *Enterobacter* are ubiquitous and are very well adapted to the environment in healthcare institutions [7]. *Streptococcus pyogenes* is a Gram positive, non-spore forming, facultative anaerobic bacteria that is capable of invasion through broken skin or mucous membrane. It causes pharyngitis, localized skin infections, rheumatic fever, rheumatic heart disease and streptococcal toxic shock syndrome [8]. *Streptococcus pyogenes* or Lance-field group A beta-hemolytic *Streptococcus* (GAS) is the commonest bacterial pathogen that causes acute pharyngitis among school-aged children living in lower socio-economic conditions [9]. The spread and virulence factors related with the various strains of GAS are not stable over time [10]. Pharyngitis is a sore throat caused by inflammation of the throat. Throat may be scratchy and swallowing can be painful. Usually, a sore throat is the sign of another illness, such as a cold or the flu [11]. With increasing severity, there may be severe pain that increases on swallowing plus cervical lymphadenopathy with or without fever [11]. *Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus* and *Enterobacter bugandensis* (secondary pathogens); *Klebsiella pneumoniae,* and *Escherichia coli* (primary pathogens have been previously reported by **Karchmer**, [12] to be culpable in respiratory tract infections.

Antibiotics are secondary metabolites produced by a variety of microorganisms and are used as antimicrobial chemotherapeutic agents [13]. Among all interventions in medicine, antibiotics have been outstandingly useful and life-saving as they have significantly led to the reduction of death rate from all types of bacterial infections [14]. The major modes of antibiotic mechanisms of activity are interference with cell wall synthesis, inhibition of protein synthesis, interference with nucleic acid synthesis, inhibition of a metabolic pathway and disorganizing of the cell membrane [15,16].

The rate of bacterial resistance to antibiotics is increasing daily. The major reason for the emergence of bacteria resistance is the misuse and overuse of antibiotics used for the treatment of infections [16]. Other reasons include the production of biofilms during quorum-sensing-regulated mechanism which releases beta-lactamase responsible for degradation of various antibiotics [17]. This study aims to investigate the prevalence and antibiotic susceptibility pattern of bacteria involved in throat infections in the study area. The molecular dynamics of clinically-significant Gram negative bacteria associated with lower respiratory tract infections was also ascertained

Materials and Methods

Study area

This study was carried out in Akure, the Ondo State capital, Nigeria. Samples were collected from the ear, nose and throat (ENT) Department of the University of Medical Sciences Teaching Hospital (UNIMEDTH) Annex (formally Ondo State Specialist hospital), Akure, Ondo State, Nigeria. The hospital is located at 7.077499 N, 4.808039 E, Akure, Ondo State capital which covers an area of 14, 798.8,993.7 square kilometres. It lies at latitude 7º15’0” N, 70 11’N 5º11’42” E and longitude 5º11’42’E, 5º35’E [18]. UNIMEDTH is a specialist hospital visited by people all over Ondo State and neighbouring towns for treatment.

Ethical approval

Ethical approval is procured for the collection of throat swab samples of patients with throat infections from Ondo State Ministry of Health, Akure, Nigeria. The document contained an Health Research Ethics Committee assigned number NHREC/18/08/2016 and protocol number OSHREC/13/11/2018/070. Informed consent was obtained from the patients prior to sample collection and administration of questionnaire.

Collection of clinical samples

Two hundred (200) clinical samples were collected from patients attending the ENT (ear, nose, and throat) Department of the University of Medical Sciences Teaching Hospital Akure, Ondo State by

the medical practitioners of the department and transported in ice packs within 2 hours of collection to the laboratory of Microbiology Department of the Federal University of Technology, Akure. Clinical samples were collected over a period of 4 months (December, 2018-March, 2019) [19].

Isolation of bacterial isolates from throat swab samples

The collected throat swab samples were aseptically spread on sterile chocolate agar plates and were incubated at 37 ℃ for 24-48 hours in 5% CO_2 to characterize haemolytic bacteria [20]. After inoculation, the plates were incubated at 37 ℃ for 24 hours in an incubator (Gallenkamp, Model IH-150 England). After incubation, each of the different colonies of bacterial growth were picked and inoculated again on separate sterile plates and incubated at 37℃ for 24 hours. The throat swabs were also streaked on MacConkey agar and Eosine methylene blue agar (Hi-Media, India) to characterize Gram negative bacteria. This process was repeated until pure cultures were obtained. Each of the pure cultures obtained were sub-cultured on sterile nutrient agar slants and stored at 4 ℃ prior to further analysis [21].

Colonial and morphological characteristics of bacteria isolated from throat swabs

The colonial and morphological characteristics of the colonies such as colour, elevation, texture and opacity were used as the first preventive test for the identification of bacterial isolates from throat swab samples [22].

Biochemical characteristics of bacteria isolated from throat swab samples

The biochemical tests carried out on fresh cultures of the test isolates include Gram staining, haemolysis, catalase, coagulase, indole, citrate, methyl red, voges-proskauer, oxidase and triple sugar iron tests. The tests were done according to the method described by **Bunyan et al.** [22], with slight modifications.

Molecular identification of bacterial isolates from throat swabs

Single colony of the bacterium to be identified was picked and cultured in a sterile nutrient broth in a test tube.

DNA extraction of bacteria isolates

Genomic extraction and purification of Deoxyribonucleic Acid (DNA) is virtually the basis of all procedures in forensic molecular biology. DNA extraction method used and DNA concentration were evaluated through the steps described by Jena bioscience Kit™ (Thuringia, Germany). The steps were classified into three (3) which are lysis, precipitation and hybridization as demonstrated by **Bunyan et al.** [22].

PCR amplification

Polymerase chain reaction was carried out using the method described by **Abayasekara et al.** [23]. The 16SrRNA gene of the bacteria was amplified using the primer pair 27F-5′ AGAGTTTGATCCTGGCTCAG-3', and 1492R 5'GGTTACCTTGTTACGACTT-3'. Thermal cycling was conducted in an eppendorf vapo protect thermal cycler (Nexus Series) for an initial denaturation of 95 °C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95 °C; 1 minute at 61 °C and 1 minute 30 Seconds at 72 °C. This was followed by a final extension step of 10 minutes at 72 °C. The amplification product was separated on a 1.5% agarose gel and electrophoresis carried at 80 V for 1 hour 30 minutes. After electrophoresis, DNA bands were visualized by ethidium bromide staining. 100bp DNA ladder (Solis Biodyne) was used as DNA molecular weight marker.

Sequencing and blasting

Polymerase chain reaction products were purified with Exo sap and then subjected to DNA Sanger sequencing and data was analyzed by ABI Sequencing Analysis software (version 5.2). The basic local alignment search tool was carried out using the NCBI (National Commission for Biotechnological Information) gene bank as conducted by **Abdulkasim et al.** [24].

Standardization of bacterial isolates from throat swab samples

The standardization of the stored pure cultures of bacterial isolates from throat swab samples was carried out by diluting a 6 hours old broth cultures of the bacterial isolates in test tubes and comparing with a 0.5 McFarland Standard to adjust their suspension to a density equivalent to approximately 10^8 CFU/ml as conducted by **Bayode et al.** [20]; **CLSI,** [21].

Antibiotic susceptibility testing of clinical and typed bacterial isolates from throat swab samples

The antibiotic susceptibility testing was done using Kirby-Bauer disc diffusion method as described by **Silverman et al. [25**] with slight modifications.

Results

Frequency of throat pathogen carriers based on age and gender

The highest frequency of carriers of throat pathogens was obtained among male patients between ages 31 and 40 years. This age range is mainly adults especially within the ages of 31-40 years old **(Figure 1)**.

Presumptive identification of bacterial isolates from throat swab samples

The bacteria recorded in this study to be associated with throat infections are *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter bugandensis* and *Escherichia coli* **(Table 1)**.

Molecular identity of *Enterobacter bugandensis* from throat swabs

The gel electrophoresis of 16S rRNA gene amplification of *Enterobacter bugandensis* strain MH712497.1 with amplicon size of 1kbp is displayed in **figure (2)**.

Percentage occurrence of bacteria isolated from throat swabs

The percentage occurrence of the bacteria isolated from throat samples of infected patients are; *S. pneumoniae* (28%), *S. pyogenes* (23%), *S. aureus* (21%), *K. pneumoniae* (15%), *E. bugandensis* (9%) and *E. coli* (4%) as shown in **figure (3)**.

Comparative antibacterial activities of some conventional antibiotics on clinical and typed isolates from throat swab samples

Staphylococcus aureus was resistant to most of the conventional antibiotics (gentamicin and amoxicillin). *Klebsiella pneumoniae* was highly susceptible to ciprofloxacin (23.00±0.58 mm), chloramphenicol (22.33±0.33 mm) and augmentin (23.33±0.88 mm). All tested Gram-positive bacteria were resistant to gentamycin (30 µg). *Streptococcus pyogenes* was resistant to gentamycin (30 µg), erythromycin (10 µg) and susceptible the other antibiotics. Isolates of *K. pneumoniae* were resistant to gentamycin (30 µg) as demonstrated in **tables (2a and 2b)**.

Figure 1. Frequency of throat pathogens' carriers in relation to age and sex distribution.

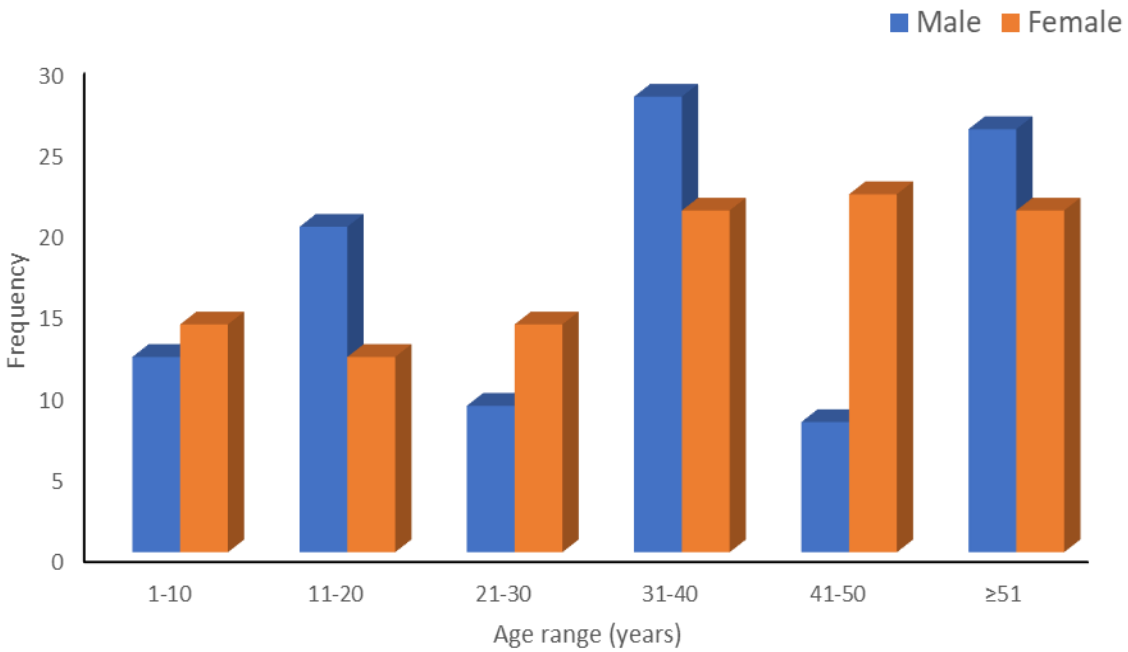
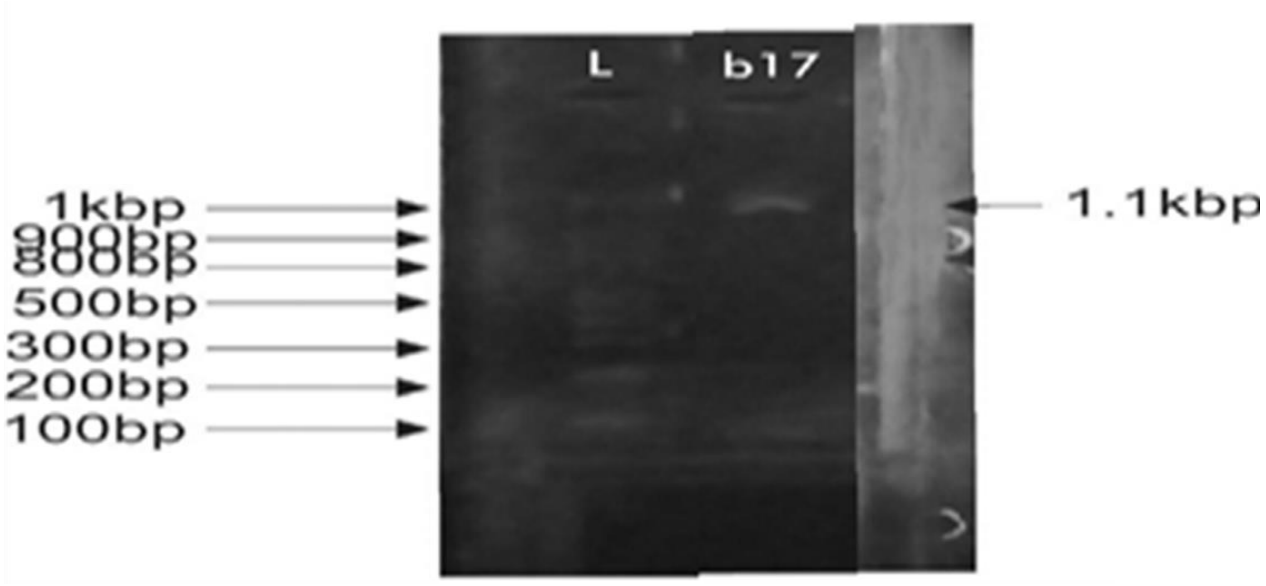


Figure 2. Gel electrophoresis picture of 16S rRNA gene amplification of *Enterobacter bugandensis* strain MH712497.1 with amplicon size of 1kbp.



Key: L= DNA ladder, b17= sample of *E. bugandensis* strain MH712497.1, bp= base pair

Figure 3. Percentage occurrence of bacteria isolated from throat swabs.

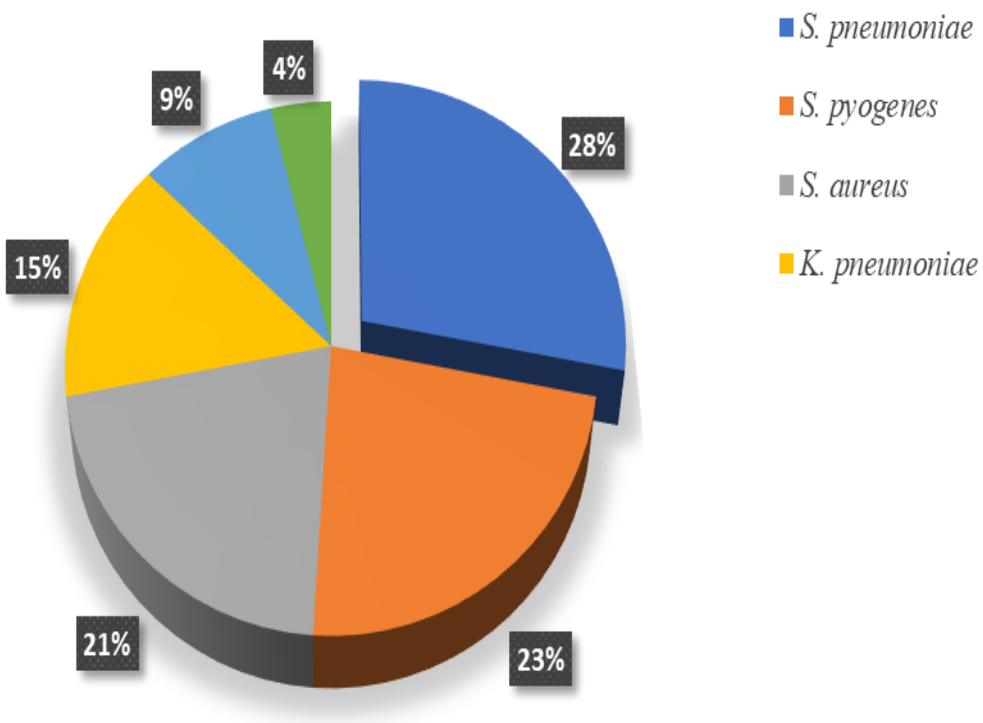


Table 1. Biochemical characteristics of isolates from throat samples.

Isolates	Colony characteristic	Gram's reaction	Catalase	Coagulase	Citrate	Oxidase	Motility	Glucose	Fructose	Lactose	Sucrose	Maltose	Probable bacteria
1	Colourless, raised	+ve cocci chains	-	-	-	-	NM	AG	AG	AG	AG	AG	*S. pneumoniae*
2	Round, hemolytic,	+ve cocci chains	-	-	-	-	NM	AG	A	AG	A	AG	*S. pyogenes*
3	Yellow, hemolytic	+ve cocci clusters	+	+	+	-	NM	AG	AG	AG	AG	AG	*S. aureus*
4	White, round	-ve rod	+	-	+	-	NM	AG	AG	AG	AG	AG	*K. pneumoniae*
5	Mucoid, smooth	-ve rod	+	-	+	-	M	AG	-	-	-	-	*Enterobacter* spp
6	Colourless, opaque, round	-ve rod	+	-	-	-	M	AG	AG	AG	AG	AG	*E. coli*

Key: NM: Non motile, M: Motile, AG: Acid and Gas production, A: Acid production only, +: present, -: absent, +ve: Gram positive, -ve: Gram negative.

Table 2a. Comparative antibacterial activities of some conventional antibiotics on Gram positive clinical and typed isolates involved in throat infections.

Antibiotics	***S. pyogenes* ATCC 12384**	***S. pyogenes***	***S. aureus* NCTC 6571**	***S. aureus***
CN S = ≥15 I =13-14 R = ≤12	16.33 ± 0.33^{a} (R)	0.00 ± 0.00^{b} (R)	17.00 ± 0.58^{a} (R)	0.00 ± 0.00^{b} (R)
AM S = ≥17 I =14-16 R = ≤13	12.00 ± 0.58^{b} (R)	20.33 ± 0.33^{a} (S)	0.00 ± 0.00^{c} (R)	0.00 ± 0.00^{c} (R)
CPX S = ≥21 I =16-20 R = ≤15	14.33 ± 0.33^{c} (R)	20.00 ± 0.58^{a} (I)	17.00 ± 0.58^{b} (I)	21.00 ± 0.58^{a} (S)
S S = ≥15 I =12-14 R = ≤11	16.00 ± 0.58^{b} (I)	20.00 ± 0.58^{a} (S)	15.67 ± 0.33^{b} (I)	21.33 ± 0.33^{a} (S)
E S = ≥23 I =14-22 R = ≤13	23.33 ± 0.33^{a} (S)	0.00 ± 0.00^{c} (R)	21.33 ± 0.33^{b} (I)	20.67 ± 0.33^{b} (I)

Key: CN: Gentamycin (30 µg), AM: Ampicillin (30 µg), CPX: Ciprofloxacin (30µg), S: Streptomycin (30 µg), E: Erythromycin (10 µg), (S): Sensitive, (R): Resistant, (I): Intermediate

Values are presented as mean±SE of duplicates, values in the same row carrying same superscript are not different significantly ($p<0.05$) according to new Duncan's Multiple Range test. S-Susceptible; I-Intermediate; R-Resistant.

Table 2b. Comparative antibacterial activities of some conventional antibiotics on Gram negative clinical and typed isolates involved in throat infections.

Antibiotics	***K. pneumoniae* ATCC 13888**	***K. pneumoniae***	***E. coli* ATCC 25922**	***E coli***
CN S = ≥15 I =13-14 R = ≤12	19.00±0.58^{b} (S)	13.67±0.33^{c} (I)	21.67±0.33^{a} (I)	20.67±0.33^{a} (I)
AM S = ≥17 I =14-16 R = ≤13	20.00±0.58^{a} (S)	0.00±0.00^{b} (R)	19.67±0.58^{a} (S)	0.00±0.00^{b} (R)
CPX S = ≥26 I =22-25 R = ≤21	21.00±0.58^{b} (S)	23.00±0.58^{a} (S)	22.33±0.33^{ab} (S)	15.67±0.33^{c} (R)
S S = ≥15 I =12-14 R = ≤11	22.00±0.58^{a} (S)	16.33±0.33^{c} (S)	20.67±0.33^{b} (S)	17.33±0.33^{c} (S)
CH S = ≥18 I =13-17 R = ≤12	22.33±0.33^{b} (S)	22.33±0.33^{b} (S)	25.00±0.58^{a} (S)	20.00±0.58^{c} (S)
AU S = ≥18 I =14-17 R = ≤13	25.33±0.33^{ab} (S)	23.33±0.88^{b} (S)	27.00±0.58^{a} (S)	26.00±0.58^{a} (S)

Key: CN: Gentamycin (10 µg), AM: Amoxicillin (30 µg), CPX: Ciprofloxacin (10 µg), S: Streptomycin (30 µg), CH: Chloramphenicol (30 µg), AU: Amoxicillin/clavulanic acid, (20/10 µg), (S): Sensitive, (R): Resistant, (I): Intermediate.

Discussion

The prevalence and antibiogram profile of bacteria associated with throat infections was divulged in this study. The outcome of the age and gender distribution of throat pathogen carriers corroborates with the findings of **Moirangthem and Gurung,** [26] that symptomatic throat infections were seen more in adults (78.18%) than children (21.82%). This may be due to the development of such infections in adult as mainly secondary infections.

Pathogenic bacterial organisms isolated from the throat swabs of infected patients agree with the findings of **Moirangthem and Gurung,** [26], **Anitha et al.** [1], **Ahmad et al.** [27], that bacteria are commonly implicated in throat infections. **Obiajurn and Chukuezi,** [19] has also reported that bacterial species such as *S. aureus, S. pneumoniae, Proteus spp.* and *Haemophilus* species are responsible for most cases of ENT infections in Orlu, Imo State, Nigeria while **Moirangthem and Gurung,** [26] recorded that *S. aureus, S. pyogenes, Pseudomonas aeruginosa* and *Proteus* spp are responsible for throat infections in a Referral Hospital in Sikkim, India.

Enterobacter bugandensis have been reported to be associated with severe clinical infections according to **Pati et al.** [7], highly pathogenic specie of the genus *Enterobacter*. It was reported as a nosocomial pathogen capable of causing life-threatening infections in neonates and immunocompromised patients. *E. bugandensis* is a Gram-negative motile rod and facultative aerobe that grows optimally at 37 ºC as reported by **Mahendran et al.** [28]; **Urban et al.** [29].

Streptococcus species had the highest percentage occurrence amidst the bacteria isolated from throat swabs in this study. This corroborates the findings of **Anitha et al.** [1] that *S. pyogenes* had the highest occurrence of bacteria isolated from throat swabs of patients attending the ENT and out-patient

department of Shri Sathya Sai Medical College and Research Institute, Thiruporur, Sri Balaji Vidyapeeth University, Tamil Nadu, India. This may be attributed to the ability of these bacteria to strive well in the mucus membranes and in air droplets according to **Anitha et al.** [1]; **Ahmad et al.** [27].

In this study, *Escherichia coli* cultures were susceptible to augmentin (10 µg). This agrees with the work of **Mekonnen et al.** [30] where augmentin was reported to be the most useful drug for the treatment of *E. coli* infections such as urinary tract and diarrhea. Antibiotics are secondary metabolites produced by a variety of microorganisms and are used as antimicrobial chemotherapeutic agents as demonstrated by **Neu and Gootz,** [13]. Both *E. coli* and *E. bugandensis* were resistant to amoxicillin (30 µg). This is similar to the report of **Aghemwenhio et al.** [31] that *Enterobacter* species possess intrinsic resistance to amoxicillin. This is because of the production of chromosomally encoded, inducible Amp C ß-lactamase **Muhammad et al.** [32]. The antibiotic susceptibility pattern obtained in this study reveals that *S. aureus* was resistant to pefloxacin (30 µg), ampiclox (30 µg), amoxicillin (30 µg), rocephin (25 µg) and gentamycin (30 µg) used in this study. This is in agreement with the reports of **Wang et al.** [6]; **Aghemwenhio et al.** [31] who both stated that *S. aureus* isolated from throat swabs and wounds respectively were resistant to β-lactam antibiotics, macrolides and aminoglycosides. This may be as a result of the presence of various resistant genes in most strains of *S. aureus* such as *mec* and *ccr* genes as reported by **Lowy,** [33]; **Chambers,** [34]; **John et al.** [35]. The *S. pyogenes* isolates tested in this study were resistant to ampiclox (30 µg) and amoxicillin (30 µg). This corroborates with **Rasheed,** [36]; **Kebede et al.** [37] that *S. pyogenes* isolates from ponds were found to be resistant to ampiclox and amoxicillin. This could be as a result of indiscriminate use of these antibiotics by patients attending the hospital.

Conclusion

This study revealed that throat infections are common among adults between ages 31 to 40 years old. *Streptococcus* species had the highest occurrence of the bacterial isolates from throat swabs of infected patients. *Enterobacter bugandensis*, a nosocomial pathogen, capable of causing life-threatening infections was among the bacteria associated with throat infections. Gram-negative bacteria tested were resistant to amoxicillin (30 µg). Finally, some of the conventional antibiotics are still effective against bacteria involved in throat infections. The presence of the novel *Enterobacter bugandensis* amidst throat infection carriers further emphasized the need for the regulation of over-the-counter prescription and survey of antienterococcal in hospital setting.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Fiancial disclosure: None.

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