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Distribution of Bacteria Species Isolated from Respiratory Tracts of Secondary School Students in Ebonyi North

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Abstract **Original Research**

Respiratory tract infection is an illness that can affect both the upper and the lower respiratory tract. Bacteria are the second most common causes of respiratory tract infections after viruses. This study employed a cross sectional study design, aimed to identify dominant bacteria species in respiratory tract of apparently healthy secondary school students in Ebonyi North. A total of 192 sputum specimens were collected randomly from secondary students of Ebonyi North Zone. *Samples were collected from both day-resident and boarding-resident secondary schools in Ebonyi North*. The sputum specimens were cultured on chocolate and blood agar and identified using standard microbiology techniques. Out of the 192 students sampled, 37.5% (72/192) were infected. A total of 6 bacterial species were identified including; *Serratia liquefaciens* (41.7%), *Proteus vulgaris* (19.4%), *Klebsiella pneumoniae* (13.8%), *Bacillus thuringiensis* (16.7%), *Staphylococcus auerus* (5.6%) and *Streptococcus pyogenes* (2.8%). Females students, 38/72 (52.8%) more infected by the bacterial spp than males 34/72 (47.2%) in this study. *S. liquefaciens* (52.6%) significantly infected more females than in males (29.4%), whereas *P.vulagaris* (23.5%), *Bacillus thuringiensis* (23.5%) and K. *pneumoniae* (17.6%), were more in males than in females. On the other hand, *S. pyogenes* only infected females. The distribution of bacteria species between the age groups of 15-16 (54%) and 17-18 (46%) years differ non-significantly. Boarding students were more infected with bacterial species than those of day residence students. This study revealed rare bacterial species implicated in respiratory tract infection especially among boarding secondary school students.

Key words: Molecular identification, Respiratory tract infection, Bacterial species.

INTRODUCTION

Respiratory tract infections (RTIs) are illnesses that affect the respiratory system (Calderaro *et al*., 2022), which spans from asymptomatic or mild infection to severe or fatal disease. The host, the ambient conditions, and the causative agent usually determine the clinical spectrum (Calderaro *et al*., 2022). Bacterial infections are the second most common cause of RTIs, behind viruses (Paul.,2024). The respiratory tract infection involves the upper and the lower respiratory tract.Upper respiratory tract infections (URTIs) often have a brief, mild, and self-limiting course (Wang *et al*.,2021). Viruses usually

target the human respiratory tract and cause a variety of clinical symptoms, including acute respiratory distress syndrome (ARDS), are typically responsible for the high prevalence of URTIs (Dominguez *et al*., 2019, Hashmi *et al*.,2021, Clementi *et al*., 2021). There are array of microorganisms implicated in respiratory tract infections with associated health implications. Some of them has been neglected and no attention paid to it especially in the tropical Africa including Nigeria and Ebonyi State. Some of the hostel environment imposes risks to disease and infection. Some of the predisposing risks factors include; exposure to cold, overcrowded hostels, poor sanitation and personal hygiene. However, some of these organisms especially in healthy young individuals can be opportunistic when predisposing factors are present. Therefore, this study aim to identify bacteria and antibiotic susceptibility in respiratory tract infection of secondary school students in Ebonyi north.

MATERIALS AND METHODS

Study Area and Sample Collection

This cross sectional study was carried out in Ebonyi north senatorial zone. This study was approved by the Ethical committee of Ebonyi State ministry of Health. A total of 192 sputum specimens were collected randomly from two secondary schools in Ebonyi north senatorial zone including boarding and day-secondary school students.

Ethical Consideration

Ethical approval was gotten from the Government of Ebonyi State Ministry of Health Directorate of Health Research and Ethical Committee with the assigned number 2025/0037(EBSHREC/037).

Isolation and Identification of Bacteria

The sputum were cultured in on blood agar and chocolate agar (Titan Biotech Ltd Rajasthan India, LOT/batch No MG3ECYO1). The bacteria were identified by standard microbiological and biochemical methods, such as Gram staining, catalase. The isolated were stored by sub-culturing into brain heart infusion broth (maunfactured by Sifin diagnostics gmbh Berliner Allee 317-321 13088 Berlin, Germany).

RESULTS AND DISCUSSION

Table 1: Occurrence of Respiratory Tract Infections among Senior Secondary School Students in Ebonyi North (n=192)

	Number of Subjects	Occurrence (%)
Infected	72	37.5
Uninfected	120	62.5

Table 2: Distribution of Bacterial species among Subjects (n=72)

Bacteria isolate	No of subjects infected	%Prevalence
Serratia liquefaciens	30	41.7
Proteus vulgaris	14	19.4
Bacillus thuringiensis	12	16.7
Klebsiella pneumoniae	10	13.8
Stapylococcus aureus	4	5.6
Streptococcus pyogenes	2	2.8

Table 3: Distribution of Bacterial spp among Subjects based on Age group

Age group	Infectious agent	Number	Prevalence (%)
15-16 (n=39)	*S. liquefaciens*	20	51.3
	P. vulgaris	5	12.8
	Klebisella	6	15.4
	pneumoniae	4	10.3
	Staphylococcus	2	4.8
	auerus	2	5.1
	B. thuringiensis		
	Streptococcus		
	pyogenes		
17-18 (n=33)	*S. liquefaciens*	10	30.3
	B. thuringiensis	10	30.3
	P. vulgaris	9	27.3
	Klebisella pneumoniae	4	12.1

Table 4: Distribution of Bacterial spp among Genders

Gender	Microbial Agent	Number Infected	Prevalence (%)
Males	*Serratia liquefaciens*	10	29.4
(n=34)	*Proteus vulgaris*	8	23.5
	Bacillus thuringiensis	8	23.5
	Klebsiella pneumoniae	6	17.6
	Streptococcus pyogenes	0	0.0
	Staphylococcus auerus	2	5.9
Females	*S. liquefaciens*	20	52.6
(n=38)	*P. vulgaris*	6	15.8
	B. thuringiensis	4	10.5
	Klesbsiellae pneumoniae	4	10.5
	Streptococcus pyogenes	2	5.3
	Staphylococcus auerus	2	5.3

Table 5: Distribution of Bacterial spp based on Residence Status

Residence status	Microbial Agents	Number	Prevalence (%)
Day (n=28)	*Serratia liquefaciens*	10	35.7
	Klebsiella	6	21.4
	pneumoniae	4	14.3
	Proteus vulgaris	4	14.3
	Bacillus thuringiensis	2	7.1
	Streptococcus	2	7.1
	pyogenes		
	Staphylococcus		
	auerus		

Boarding (n=44)	*Serratia liquefaciens*	20	45.5
	Proteus vulgaris	10	22.7
	Bacillus thuringiensis	8	18.2
	Klebsiella pneumoniae	4	9.1
	Staphylococcus auerus	2	4.5

Table 7: Distribution of Bacterial Isolates based on Age and Residence

Age group (years)	Residence	Isolates	Number (%)
15-16	Day (n=11)	*Serratia liquefaciens*	6 (54.5%)
		Klebsiella pneumoniae	2 (18.2%)
		Bacillus thuringiensis	1(9.1%)
		Proteus vulgaris	1(9.1%)
		Staphylococcus auerus	1(9.1%)
	Boarding (n=25)	*Serratia liquefaciens*	14 (56%)
		Proteus vulgaris	4 (16%)
		Klebsiella pneumoniae	4 (16%)
		Streptococcus pyogenes	2 (8%)
		Bacillus thuringiensis	1(4%)
17-18	Day (n=14)	*Serratia liquefaciens*	4 (28.6%)
		Proteus vulgaris	3 (21.4%)
		Bacillus thuringiensis	3 (21.4%)
		Klebsiella pneumoniae	4 (28.6%)
	Boarding (n=22)	*Serratia liquefaciens*	6 (27.3%)
		Proteus vulgaris	6(27.3%)
		Bacillus thuringiensis	7 (31.8%)

Staphylococcus aureus	3(13.6%)
Klebsiella pneumoniae	0 (0%)

DISCUSSION

This study was aimed at investigation bacteria diversity and associated health impacts in respiratory tract infections of secondary school students in Ebonyi north. The result of table 1 shows a total of 192 samples were collected. Among this, only 37.5% were infected the other 62.5% were either contaminated or had no growth. The infecting microorganisms were *Serratia liquefaciens*, *Proteus vulgaris*, *Bacillus thuringiensis*, *Klebsiella pneumoniae*, *Staphylococcus auerus* and *Streptococcus pyogenes*. The most dorminants were *Serratia liquefaciens*, *Proteus vulgaris*, *Bacillus thuringiensis*, *Klebsiella pneumonia*.

The most often isolated species of the genus Serratia from clinical specimens, particularly healthcare-associated illnesses (HCAIs), is *Serratia marcescens*, which belongs to the order Enterobacterales (Williams *et al*.,2022). Most human Serratia infections are linked to the *S. marcescens* and *Serratia liquefaciens* complex, which includes *S. liquefaciens*, *Serratia proteamaculans*, and *Serratia grimesii*. *S. liquefaciens* are rare and opportunistic infection and healthy people are rarely infected by *S. liquefaciens* except hospitalized patients, those with compromised immune system, those with poor living condition, those with chronic respiratory disease and those with indwelling/introduced foreign bodies/liquids, e.g.,intravenous/intra-arterial (IV/IA) lines, endotracheal tubes, multiple use vials; thus, the entry routes are ingestion, injection and catheterization (Ikumapayi *et al*., 2016).This study established the presence of *S.liquefaciens* (41.7%)in the respiratory tract. This is in line with the study carried out by Ikumapayi *et al*., 2016 which identified *S. liquefaciens* in the lung aspirate of those with pneumonia. According to the study carried out by Belheouane *et al*., 2025. *Serratia liquefaciens*, *Serratia grimesii*, *Serratia myotis*, or *Serratia quinivorans* were found in TB and non-tuberculous mycobacteria lung diseases (NTM-LD) patients, according to exploratory whole-metagenome sequencing and amplicon sequence variants similarity studies.

Proteus vulgaris was the second most dormant bacteria (about 19.4%) identified by this study. It is a Gram negative bacteria mainly known to cause urinary tract infections, but it is becoming more well acknowledged as an opportunistic pathogen in respiratory tract infections (RTIs), particularly in animals and immunocompromised hosts. Its virulence mechanisms, increasing antibiotic resistance, and presence in respiratory samples from both humans and animals are highlighted in recent investigations (Rahman *et al*.,2025). This finding is in line with the study conducted by Fouad *et al*.,2023, *Proteus vulgaris* was found in 4 out of 91 positive microbiological isolates in a research of respiratory tract infections carried out in Great Cairo from January to June 2022, indicating a frequency of 4.4% among the cultured samples. Another study conducted by Shah *et al*.,2016 identified *P.vulgaris* in the sputum samples of patients in a study of RTIs in North Waziristan, Pakistan. *P.vulgaris* accounted for 4.6% of bacterial isolates, although less prevalent as compared with *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, *Proteus vulgaris* is a significant pathogen.

Bacillus thuringiensis from this study showed about 16.7% of the bacterial population. *Bacillus thuringiensis* is regarded as non-infectious for healthy persons and is frequently employed as a biopesticide. However, research on farm and greenhouse workers exposed to *Bacillus thuringiensis* sprays has demonstrated that inhalation can result in immune sensitization, with elevated IgE and IgG antibodies against *Bacillus thuringiensis* and positive skin-prick tests, but no

appreciable increase in respiratory symptoms or measurable impairment of lung function in healthy individuals (Bernstein et al., 1999, Baelum et al., 2012). Studies by Ghelardi *et al*., 2007 and Belousova *et al*., 2021 has shown that *Bacillus thuringiensis* can infect the lungs of immunocompromised hosts, even though this is uncommon. According to the animal studies neutropenic mice are extremely vulnerable to deadly *Bacillus thuringiensis* pneumonia, and membrane-damaging toxins are vital for persistence and spread. Reports of *Bacillus thuringiensis* infection in humans are extremely rare and usually entail underlying immune suppression. There have not been any notable study specifically addressing *Bacillus thuringiensis* in human respiratory tract.

From the result of table 2, *Klebsiella pneumoniae* is the fourth most prevalent microorganism having about 13.8% of the microbial population. *Klebsiella pneumoniae* was identified as a microorganism in the respiratory tract. Although it has been known to be an opportunistic bacteria but can result in pneumonia when there is suppression of immune system or predisposing factors. The finding is in line with the finding of Li *et al* 2025 which identified *K. pneumonia* as a global burden and associated with respiratory tract infections particularly lower respiratory tract infection.

Staphylococcus auerus and *Streptococcus pyogens* (5.6% and 2.8% respectively) was also isolated in this study. This is line the study of Hussein 2024, which showed 29.166% of *Stapylococuss aureus* and 39.500% *Streptococcus pyogenes* in the sputum of patients with respiratory tract infection.

Females (38(52.7%) have been found to be more susceptible than males 34(47.2%) in this study. All the bacterial species implicated in this study were all present in the females whereas all microbial species excluding *Streptococcus pyogenes* were present in the males. This finding is in line with the findings of Ashiq *et al*, in which females were more susceptible to bacteria infection in the respiratory tract. However it is contrary to the findings of Falagas *et al*,Dias et al and Peer *et al* that males are more susceptible to respiratory tract infections infections than females. The finding of this study showed that students of boarding residence had more microbial infection than students of day residence. This could be due to overcrowding, frequent person to person, poor environmental and personal hygiene and lack or reduced ventilation in poor designed hostels. This finding is in line with the study of Yang *et al* which showed that boarding students often experience a higher prevalence of respiratory tract infections (RTIs) compared to day students.

This study showed that in table 3, the distribution of infectious agents were more between the age group of 15-16 than between the age group of 17-18. *Streptococcus pyogene* and *Staphylococcus auerus* were not seen in the age group 17-18 but were seen in the age group of 15-16. the finding of this study also showed that *S. liquefaciens* (52.6%) were more in females than in males whereas *P.vulagaris* (23.5%), *Bacillus thuringiensis* (23.5%) and *K. pneumoniae* (17.6%), were more in males than in females while *S. pyogenes* were not seen in males (table 4). This also reviewed that there were more microorganisms in subjects of boarding residents than the day residents. *S. liquefaciens* (45.5%), *P.vulgaris (22.7%), Bacillus thuringiensis* (18.2%) were more in the boarding residence while *K. pneumoniae* (21.4%) were more in those of day residents. *Streptococcus pyogene* were only seen the subjects of day residents (table 6).

In comparing distribution of bacterial isolates based on age and residence, the finding of this study showed that boarding resident subjects with the age group of 15-16 had more *S.liquefaciens* (56%), *P. vulgaris* (16%) than subjects day residents with the same age group. Boarding residents within the age group of 17-18 had more bacteria having *S.liquefaciens* (27.3%), *P. vulgaris* (27.3%), *B. thuringiensis* (31.8%) and no *K. pneumoniae.* Day residence subjects with the age group of 17-18 had less bacterial; *S.liquefaciens (28.6*%), *P. vulgaris* (21.4%), *B. thuringiensis* (21.4%) and it has more *K. pneumoniae (*28.6*)* than boarding residents with the same age group.

Conclusion

The bacteria that are not quiet know have been overlooked by medical personnel when diagnosing

respiratory tract infections. Bacteria that are commensal can turn into opportunistic pathogens causing infections. This study has helped identified bacteria rarely known to be implicated in respiratory tract infection,which may ultimately improve patient diagnosis and treatment outcomes and consequently minimize the healthcare burden associated with pneumonia treatment and other respiratory tract infections.

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