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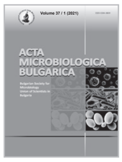

Antibiotic Resistance in Nasal Isolate of Gas Flaring Workers in a Niger Delta Region, Nigeria

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Abstract

This study aimed to determine both the bacterial species colonizing the nasal cavity and the antibiotic resistance pattern of workers at a gas flaring company in the Niger Delta Region (NDR) of Delta State on a Microbiological and questionnaire level. Nasal swab samples were collected from 20 workers at a gas flaring company and inoculated into appropriate media. Isolates were identified following standard microbiological methods while antimicrobial susceptibility testing was performed by disk diffusion method. Awareness of exposure to gas flared, attitude, knowledge, and antibiotic use were assessed with a structured questionnaire. Five bacteria species were isolated with *Staphylococcus aureus* and *Klebsiella pnuemoniae* being the most common (n = 15; 25.4%). All gram-negative organisms isolated showed 100% resistance to amoxicillin-clavulanic acid, ceftazidime, and gentamycin while greater than 80% resistance to erythromycin and ofloxacin was observed in the gram-positive isolates. A major predictor of antibiotic resistance in the NDR is PM2.5, which has led to increased resistance observed. All the respondents were aware of their exposure to flared gas and wore masks. In spite, 40% developed respiratory disease Coordinated efforts of Niger Delta Governments are required to minimize the public health risks associated with PM exposure.

Keywords: Niger Delta Region, gas flaring, antibiotics, antibiotic-resistant bacteria, air pollution

Резюме

Това проучване имаше за цел да определи както бактериалните видове, колонизиращи носната кухина, така и модела на резистентност към антибиотици на работниците в компания за изгаряне на газ във факел в района на делтата на Нигер (NDR) на щата Делта на микробиологично ниво. Взети са проби с назален тампон от 20 работници в компания за изгаряне на газ и инокулирани в подходяща среда. Изолатите са идентифицирани чрез стандартни микробиологични методи, докато тестването за антимикробна чувствителност е извършено чрез дисков дифузионен метод. Информираността за експозиция на изгорели газове, отношението, знанията и употребата на антибиотици бяха оценени със структуриран въпросник. Изолирани са пет вида бактерии, като *Staphylococcus aureus* и *Klebsiella pnuemoniae* са най-често срещаните (n = 15; 25.4%). Всички изолирани грам-отрицателни микроорганизми показват 100% резистентност към амоксицилин-клавуланова киселина, цефтазидим и гентамицин, докато над 80% резистентност към еритромицин и офлоксацин се наблюдава при грам-положителните изолати. Основен предиктор на антибиотична резистентност в NDR е PM2.5, което доведе до повишена наблюдавана резистентност. Всички респонденти са били наясно с излагането си на изгорен газ и са носили маски. Въпреки това, 40% са развили респираторни заболявания. Необходими са координирани усилия на правителствата на делтата на Нигер, за да се сведат до минимум рисковете за общественото здраве, свързани с експозицията на прахови частици.

Introduction

The world is gradually transiting into a post-antibiotic era in which antibiotic-treatment failure and mortality caused by bacterial infections are increasing (Brown and Wright, 2016). Developing countries in Africa are overburdened with the negative effects of antimicrobial resistance

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(Ayukekbong *et al.,* 2017; Morel *et al.,* 2020) with over 700 000 deaths annually and an estimated 4.1 million deaths by 2050 (Dadgostar, 2019). It is increasingly recognized that tackling the matter of the overuse or misuse of antibiotics in hospitals alone would not be sufficient to control antimicrobial resistance because the spread of resistant strains and resistance genes is a dominant contributing factor (Collignon *et al.,* 2018). Besides, the one health approach recognizes that animal, human, and environmental health are interconnected.

Airborne bacteria and Antibiotic resistance genes are emerging environmental pollutants, with strong evidence showing that increasing levels of air pollution are associated with increased risk of antibiotic resistance (Wang *et al.,* 2019). With the extensive use of antibiotics in human and animal medicine, the atmosphere has become an important vector and reservoir for antibiotic resistance genes and is a gateway to potential health risks through inhalation and ingestion.

Air pollution from gas flaring has a substantial effect on the environment and has become a serious health threat to individuals working or living near a flared well. Pollution of air from gas flaring is a daily occurrence in the Niger Delta Region (NDR) because it is part of the conventional oil production. The air quality of nine-tenths of the Niger Delta people especially those living in the areas where gas is flared is above recommended levels (Nwachukwu *et al.,* 2012; Ubong *et al.,* 2015; Yakubu, 2018; Kalagbor *et al.,* 2019).

Flaring releases high concentrations of complex mixtures of gases and particulate matter (PM) 2.5(Wang *et al.,* 2019). Exposure to air pollutants induces oxidative stress and inflammation in the airways and is widely believed to trigger and exacerbate many respiratory diseases (Kelly and Fussell, 2011; Arbex *et al.,* 2012).

Studies have shown the involvement of the respiratory microbiota in the maintenance of homeostatic functions in the airway and modified by exposure to air pollution (Man *et al.,* 2017). Air pollution is aiding the rise in antibiotic resistance, as resistant bacteria and resistance genes in hospitals could be transmitted to ecosystems, and be exposed to humans through inhalation(Zhu *et al.,*2021; Jin *et al.,*2021).

The particulate matter (PM) 2·5 is a main component of air polluted by gas flares and has been shown to contain diverse antibiotic-resistant bacteria and antibiotic-resistance genes, which are transferred between environments and directly inhaled by humans. (Xie *et al.,* 2019; Wang *et al.,* 2019). Thus far, no study has attempted to associate antibiotic resistance with respiratory bacteria isolated from a population exposed to gas flaring. This study was conducted both to identify the bacterial species from the nasal swabs of workers at a gas flaring company in the NDR of Delta state and to determine their resistance pattern.

Materials and Methods

Sample collection and isolation

This study investigates the antibiotic resistance profile of isolates obtained from the nasal cavity of employees of a gas flaring company in Kwale, Delta State Nigeria. Participants were recruited between February and March 2019. All the participants were informed about the objectives of the study. Sterile swabs were used to sample the nasal cavity of the participants. The swab samples were then inoculated onto already prepared and sterilized blood agar, Eosin Methylene Blue agar, MacConkey agar, and pseudomonas agar.

Sampling technique

A simple random sampling technique was employed in this study. Twenty employees of a gas flaring company participated, they all gave consent and filled and returned the questionnaire.

Perception of air pollution, health, and antibiotic use

A structured questionnaire containing sections on sociodemographic variables and designed to meet the objectives of this research was used for data collection. The survey instrument comprised 4 questions which were on demographic information, awareness of exposure to gas flare, attitude towards wearing a nose mask, knowledge of health status, and antibiotic use. The instrument was administered to the respondents via a one-on-one interview

Determination of antibiotic sensitivity

In order to evaluate the bacterial resistance to 10 antibiotics used in this study, the agar disc-diffusion method according to Clinical and Laboratory Standard Institute guidelines (CLSI) guidelines 2018. The inoculum used for sensitivity was prepared by suspending 4-5 isolated colonies of the same morphology in 5 ml of sterile physiological saline equivalent to a 0.5 McFarland's standard which served as a reference in adjusting turbidity of bacterial suspensions (Egbule and Yusuf, 2019; Egbule, 2022).

This was then inoculated on Muller-Hinton agar (MHA) (Oxoid Ltd., England) using a sterile cotton swab. Antibiotic disks were placed and incubated at 37°C for 24 h. The zone of growth inhibition was measured and recorded as susceptible, intermediate, or resistant based on Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018). The antibiotics used were ciprofloxacin 5 µg, ceftazidime 30 µg, cefixime 30 µg, gentamicin (Gen), 10 lg; ofloxacin (Ofl), 30 µg; amoxicillin-clavulanic acid (Aug), 30 µg; nitrofurantoin (Nit), 25 µg, erythromycin (Ery), 15 µg; cefuroxime (crx), 30 µg; chloramphenicol (Chl), 30 µg.

Results

From the twenty gas-flaring workers included in the study five bacterial species were isolated from the nasal cavity of the workers. *Staphylococcus aureus* and *Klebsiella pnuemoniae*were the most common isolate (n=15; 25.4%), followed by *Streptococcus pnuemoniae* (n =11, 18.6%) then *Escherihia coli*(n = 10, 16.9%), and *Pseudomonas aeruginosa* (n = 8, 13.3%). The result is presented in Table 1.

Table 1. Bacteria isolated from gas flaring workers

S/N	Bacteria	Number (%)
1	*Escherichia coli*	10 (16.9)
2	*P. aeruginosa*	8 (13.3)
3	*K. pneumoniae*	15 (25.4)
4.	*S. pneumoniae*	11 (18.6)
5	*S. aureus*	15 (25.4)
Total		59

Information on the resistance profile of the isolates is shown in Tables 2 and 3. Most of the isolates were 100% resistant to the beta-lactam antibiotics. All gram-negative organisms isolated showed 100% resistance to amoxicillin-clavulanic acid, ceftazidime, and gentamycin. *E. coli* exhibited overall high levels of resistance wherein a 100% resistance was observed to 7 of the 8 tested antibiotics. in spite, no *E. coli* isolate was resistant to ofloxacin. However, in general, low levels of resistance (less than 40%) were observed in all gram-negative isolates to oflaxacin in this study. Low levels of less than 40% were also observed for gentamycin in the gram-positive isolates. Equally observed in the gram-positive isolates was a greater than 80% resistance to erythromycin and ofloxacin (Table 3). *S. aureus* exhibited 100% resistance to 6 of 8 antibiotics tested, including erythromycin. Additionally, all other antibiotics analyzed in this study showed high levels of resistance.

The background characteristics of the employees of a gas flaring station are presented in Table 4. The age group ranged from 15 to 54, with the majority (35%) in the age group of 35 to 44. Male respondents were more than the females. All the respondents had tertiary education and were predominantly Engineers.

With regard to knowledge of exposure to gas and their respiratory health status, all the respondents were aware of their exposure to gas flares (air pollution) and took precautions in their workplace by wearing masks (Table 5). Despite wearing masks, 25% had respiratory diseases such as asth-

Table 2. Antibiotic susceptibility pattern of Gram-negative bacteria isolated from workers of a gas flaring company

Isolates & number	Antibiotic resistance pattern (n %)							
	CPR	CAZ	CRX	GEN	CXM	OFL	AUG	NIT
E. coli (10)	1(10.0)	10 (100)	10 (100)	10 (100)	10 (100)	0 (0.0)	10 (100)	10 (100)
P. aeruginosa (8)	1 (25)	8 (100)	2 (25)	8 (100)	0 (0.0)	3 (37.5)	8 (100)	7 (87.5)
K. pneumoniae (15)	0 (0.0)	15 (100)	15 (100)	15 (100)	1 (67)	3 (20)	15 (100)	15 (100)

Key: CPR= ciprofloxacin, CAZ= ceftazidime, CRX = cefuroxime,GEN =gentamycin CXM=cefixime, OFL= ofloxacin, AUG=amoxicillin-clavulanic acid, NIT= nitrofurantoin.

Table 3. Antibiotic susceptibility pattern of Gram-positive bacteria isolated workers of a gas flaring company

Isolates & number	Antibiotic resistance pattern (n %)							
	CRX	GEN	CTR	ERY	OFL	CAZ	AUG	CXC
S. pneumonia (11)	11(100)	4 (36.4)	11(100)	11(100)	10(90.9)	11(100)	11(100)	11(100)
S. aureus (15)	15(100)	4(26.7)	15(100)	13(86.7)	14(93.3)	14(93.3)	15(100)	15(100)

Key: CRX = cefuroxime,GEN =gentamycin, CTR= ceftriaxone,ERY= erythromycin CAZ= ceftazidime,OFL= ofloxacin, AUG=amoxicillin-clavulanic acid, CXC =cloxacillin.

Table 4. Socio-demographic data

Variables (n=20) Age (years)	Male n(%)	Female n(%)	Total n(%)
15-24	1(5.0)	1(5.0)	2(10.0)
25-34	4(20.0)	4(20.0)	8(40.0)
35-44	7(35.0)	1(5.0)	8(40.0)
45-54	2(10.0)		2(10.0)
55 and above			
Total	14(70.0)	6(30)	20(100.0)
Marital status			
Living with spouse	8(40.0)	2(10.0)	8(40.0)
Living without spouse	6(30.0)	4(20.0)	12(60.0)
Highest qualification			
Primary			
Secondary			
Tertiary	14(70.0)	6(30.0)	20(100.0)
Others			
Total	14(70.0)	6(30.0)	20(100.0)
Job type			
Student	2(10.0)	1(5.0)	3(15.0)
Lab scientist	4(20.0)	3(15.0)	7(35.0)
Engineer	8(40.0)	2(10.0)	10(50.0)
Medical doctor			
Pharmacist			
Self-employed			
Other			
Total	14(20.0)	6(30.0)	20(100.0)

ma. There are likely to be other respiratory diseases because when asked if they have been diagnosed with respiratory infections as a result of exposure to gas flared, 40% of the respondents affirmed they have. On antibiotic use, 25% were admitted for respiratory infection and received antibiotics. However, all the respondents use antibiotics without a prescription (Table 6).

Discussion

Many healthy humans asymptomatically harbor potential pathogens in their respiratory tract. *S. aureus* and *Streptococcus sp.* are among the normal flora of the respiratory tract (Watson *et al.,* 2006). In this study, *S. aureus* was the most prevalent followed by S. *pneumoniae.* Other non-indigenous bacteria were isolated, these bacteria occasionally turn into pathogens causing infectious diseases when immune defenses are lost or weakened, as it is with most individuals living in polluted environments.

Table 5. Respondent awareness on exposure to gas flare and attitude towards wearing of nose masks

Survey question	Frequency (n)	Percentage (%)
Are you aware of your exposure to gas flares?		
Yes	20	100
No	0	0.0
Not sure	0	
Total	20	100.0
When working do you put on a nose mask?		
Yes	20	100.0
No	0	0.0
Not sure	0	
Total	20.0	100.0

Table 6. Respondent attitude and knowledge on health and use of antibiotics

Survey question	Frequency(n)	Percentage(%)
Do you often visit the hospital for check-up		
Yes	18	90.0
No	2	10.0
Not sure		
Total	20	100.0
Have you been diagnosed with any infection resulting from your exposure to gas flare		
Yes	8	40.0
No	11	55.0
Not sure	1	5.0
Total	20	100.0
Do you have any respiratory disease (such as asthma)		
Yes	5	25.0
No	14	70.0
Not sure	1	5.0
Total	20	100.0
Have you ever been admitted for or received treatment for respiratory diseases		
Yes	5	25.0
No	14	70.0
Not sure	1	5.0
Total	20	100.0
Have you ever been told to stay away from work because of ill health?		
Yes	10	50.0
No	10	50.0
Not sure		
Total	20	100.0

The ability of the respiratory normal flora to resist pathogen colonization plays an important role in microbial community homeostasis and airway health (Blaser and Falkow, 2009). This homeostasis may be disturbed by environmental pollutants which also promote pathogenic microbial colonization and disrupt effective nasal mucociliary clearance. This may be the cause of respiratory infections experienced by some respondents. In this study, other than the 2 Indigenous flora *Staphylococcus* and S*treptococcus* there was an increased carriage of other potentially pathogenic bacteria: *P. auerugi-nosa, K. pneumoniae,* and *E. coli*. The imbalances in the respiratory microbial community created by pollution may also have contributed to harboring other bacteria and acquisition of new bacteria genes such as antibiotic resistance genes (Murphy *et al.,* 2009).

K. pneumoniae, P. aeruginosa, and *E. coli* are the dominant pathogens in polluted air (Zhao *et al.,* 2022). The isolation of *K. pneumoniae, P. auerug-inosa,* and *E. coli,* reported as dominant pathogens in polluted air from the nasal cavities of individuals exposed to gas in this study further gave credence to the abundance of aerosolized airborne dominant pathogens in polluted air. The dynamic nature of the atmosphere could lead to an increased rate of transfer of airborne bacteria and could also pose a significant health risk to inhabitants since they can penetrate deeply into the alveolar region of human lungs along with the aerosol Xie *et al.,* 2019; Xue *et al.,* 2020; Thangavel *et al.,* 2022).

An overall high rate of antibiotic resistance observed in this study is a major concern to airways clinical practice because the antibiotics to which the bacteria showed complete resistance included the beta-lactams, amoxicillin-clavulanic acid, and erythromycin which are very strong broad-spectrum antibiotics and is in no way indicative that when infections with these organisms occur, it will be effective to treat with these agents. Studies have shown that with high levels of beta-lactamase production in *S. pneumoniae* and reduced susceptibility to beta-lactam antibiotics, treatment options shifted to fluoroquinolones and macrolides and this has resulted in increased resistance to these agents (Vardakas *et al.,* 2008; Patel *et al.,* 2011). In agreement with previous studies, *S. pneumoniae* showed 100% resistance to macrolide, and erythromycin and 90% resistance to ofloxacin.

Several studies in Nigeria and globally have shown that flared gas is composed of complex mixtures of gases and particulate matter (PM) (Nwachukwu *et al.,* 2012; Yaduma *et al.,* 2013; Giwa *et al.,* 2014; Ubong *et al.,* 2015; Guan *et al.,* 2016; Whyand *et al.,* 2018). PM 2.5 has been reported in virtually all studies on air pollution from the activities of gas flaring in the NDR A study carried out by (Eluke *et al.,* 2021) on some Niger Delta Regions of Delta State, Akwa Ibom and Bayelsa, an annual quantity of 1.29 × 104 tons of PM2.5 was reported, with figures from Delta State having significantly elevated levels of PM 2.5. PM2· 5 has been shown to contain diverse antibiotic-resistant bacteria and antibiotic-resistance genes. It is important to state that with constant flaring and serious PM 2.5 aerosol pollution, the concentration of antibiotic-resistant bacteria in the atmospheric environment is expected to be high. The inhabitants are exposed to this air daily, therefore, there is a substantial daily risk of inhalation of antibiotic resistance genes. Some reporters observed that daily inhalation of antibiotic-resistance genes exceeds the intake of antibiotic-resistance genes through drinking water (Akortha and Egbule, 2008; Egbule *et al.,* 2016a; Wang *et al.,* 2019; Li *et al.,* 2021).

The respondents all had tertiary education, which gives strength to the validity of this research and also explains the level of awareness of their exposure to gases flared (Table 4), as such took precautions by wearing masks at the workplace (Table 5). Although constant wearing of masks has no significant effect on pulmonary function in healthy people for patients with respiratory infections such as asthma, caution should be taken when using these masks. Wearing a mask for long periods tends to cause discomfort when breathing due to the accumulation of carbon dioxide and a decrease in the concentration of inhaled oxygen, which can cause an increased respiratory dead space (Egbule, 2016). Mask-wearing is likely to end at the workplace and not extended at home. The air environment of flaring sites can cross to residential quarters and beyond spreading antibiotic resistance over long distances.

In addition to particulate matter, other relevant predictors of antibiotic resistance in this study were the use of antibiotics without prescription and developing asthma from exposure to gas flared. Misuse and overuse of antibiotics is a common phenomenon in most developing countries of Africa (Egbule and Yusuf, 2017; Egbule, 2016*,* 2016b; Iweriebor *et al.,* 2021; Iweriebor *et al.,* 2022) and this exacerbates the emergence of antibiotic-resistance elements. These elements eventually get into the environment, coupled with PM 2.5 prevalent in the air of gas flared environment, which can facilitate the horizontal gene transfer of antibiotic-resistance genes between bacteria. This study therefore highlights ambient air pollution as an important antibiotic-resistance bacteria diffusion vectors and reservoirs. It is worrying to have identified that as much as 40% of the respondents developed respiratory disease as a result of exposure to the gas flared. (Table 6) and about 25% affirmed to have asthma. Respiratory infections such as asthma, bronchitis, and skin diseases among patients residing close to the flaring site have been reported in several studies in Nigeria (Ogunfiditimi, 2005; Moneke *et al.,* 2020). The health of susceptible and sensitive individuals is impacted more making those with asthma candidates for chronic asthma, other severe respiratory conditions, and high rates of hospitalization (a measurement of morbidity) (Manisalidis *et al*., 2020). This inadvertently increases antibiotic use, overuse, and abuse. Additionally, PM2.5 exposures is associated with a long recovery time and an overall medical burden (Atkinson *et al.,* 2014).

Therefore, with coordinated actions of Niger Delta governments, effective policies can be put in place to minimize the public health risks associated with PM exposure.

Conclusion

The nasal cavity of workers of a gas flaring company was found to be composed of *E. coli, P. aeruginosa,* and *K. pneumoniae*, common pathogens of air pollution by PM2.5. These colonizers can transit into infection mode when the immune system is compromised. Infections with these organisms when and if they happen will likely be resistant to most antibiotics used in this study, which are broad-spectrum first-line drugs. PM2·5 has been shown to contain diverse antibiotic-resistant bacteria and antibiotic-resistance genes. With constant inhalation by workers of gas flaring companies and inhabitants, there is bound to be increasing levels of antibiotic resistance. Additionally, this study has shown that exposure to gas flared could lead to respiratory infections, which inadvertently increases antibiotic use, overuse, and abuse. Therefore, there is a need for effective policies to minimize the public health risks associated with PM 2.5 exposure.

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