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Antibiotic Susceptibility Pattern of Pseudomonas Aeroginosa Isolated from Ready to Eat Food from Selected Street Vending Food Locations in Ikpoba-Okha Local Government Area of Edo State

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Abstract

This study was conducted to analysis of the antibiotic susceptibility pattern of *Pseudomonas aeroginosa* isolated from ready-to-eat food in selected street food vendors in Ikpoba-okha LGA. The mean total viable plate counts (TVC) for *Pseudomonas aeroginosa* was ascertained with the spread plate methods using nutrient agar with results indicating a mean total viable count (TVC) ranging from 2.20 x 10^5 to 1.05 x 10^3. The highest counts of 2.20 x 10^5 was obtained in food samples collected from SFL 5 while the lowest count of 1.05 x 10^3 was obtained from SFL 1. The organism had a high percentage occurrence of 90% haven been isolated from most food samples (especially soups) from all street food vending location except SFL 3.The data obtained from the gram negative antibiotic susceptibility analysis showed that *Pseudomonas aeroginosa* was more sensitive to Ciprofloxacin (10µg) and Norfloxacin (10µg), sensitive to Gentamycin (10µg), Augmentin (30µg), Amikacin (30µg) and Ceftazidime (30µg) but showed resistance Cotrimoxazole (30µg), Ceftriazone (30µg) and Tetracycline (30µg). The results of this study indicates that most of the ready to eat food samples examined had high contamination of *Pseudomonas aeroginosa* and hence did not meet microbiological quality standards. Hence, it is recommended that a more close supervision of ready to eat food from street food vending locations in Ikpoba-okha should be carried out by relevant authorities.

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Introduction

Ready to eat foods which are sold by different street food vendors and provide a wide range of essential energy needs and nutrients are eaten daily in Nigeria and the world over. The food are known to be very affordable for the lower and middle income groups and are appreciated for their unique flavours and convenience (Muzaffar *et al*., 2009). In contrast to these potential benefits, it is also recognized that ready to eat food are often highly contaminated by microbes as a result of food vendors who are often poor, uneducated, and

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lack knowledge in safe food handling, environment, sanitation and hygiene, mode of food display, food service and hand washing, sources of raw materials, and use of potable water. Consequently, ready to eat food are perceived to be a major public health risk (Bhowmik, 2010). Contamination of ready to eat food is as a result of so many factors such as preparation methods, poor packaging, indiscriminate waste disposal, poor sanitation, poor hygiene of food handlers, exposure of food to open air, contaminated kitchen equipment and utensils, contaminated water used in washing kitchen equipment and utensils and used for preparing food, contaminated food vending surfaces and too many people clustering around the food vending area (Omemu and Aderoju, 2008). *Pseudomonas aeroginosa* is a known microbial food contaminant which can cause foodborne diseases. It is of high public health significance haven be reported to be the second most common cause of pneumonia, the fourth most cause of urinary tract infection, and the sixth most common blood stream isolate in intensive care units (ICUs). Many potential reservoirs of the organisms have been identified including vegetables (Mahmoud *et al*., 2013). *Pseudomonas* is a Gram-negative rod, motile, aerobic Gamma-proteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species and are commonly found in soil and water and also regularly associated with the surfaces of plants and occasionally with surfaces of animals (Euzéby, 1997; Kenneth, 2008). *Pseudomonas* is an opportunistic pathogen (Igbinosa *et al*., 2011), which can cause urinary tract infections, sepsis (blood stream infection), pneumonia, pharyngitis, pulmonary disease and many other medical problems (Moore *et al*., 2008).

There has also been an observed increase in the patronage of ready to eat food vendors within Benin-City, Edo State, Nigeria (Wogu *et al*., 2011) and especially in Ikpoba-okha Local Government Area (LGA) of Edo State (Okareh *et al*., 2015) . However, their poorly regulated operations raise serious questions about food safety and hygiene standard, as well as monitoring by relevant authority (Barro *et al*., 2006; Abdalla *et al*., 2008). Ikpoba-okha is a fast growing urban centre, expanding rapidly in size and population and is characterised by people on the move; this creates a suitable environment for ready to eat food trade which unfortunately operates under unsanitary conditions. This study is aimed at analysing the public health risk of ready to eat food by carrying out an analysis of the antibiotic susceptibility pattern of *Pseudomonas aeroginosa* isolated from ready-to-eat food in selected street food vendors in Ikpoba-okha LGA.

Materials and Methods

Study Area

This study was carried out in Ikpoba-Okha local government area of Edo State, located in the South-South geopolitical zone of Nigeria. Ikpoba-Okha is a densely populated with a total population of 372,080 according to the 2006 population census conducted by the National Population Commission of Nigeria, and with an increase to 487,400 estimated projection for 2016. Ikpoba-Okha has the second LGA with the highest population (NPC 2006). The inhabitants of the area are mainly small to medium scale business owners, farmers, artisans, civil servants, bankers, and students. The people are a combination of Christians, Muslims, and traditionalists. Major languages spoken are English, Pidgin English and Edo. Ten (10) street food vending location mostly patronised by consumers in LGA were selected for this study.

Sample Collection

Three (3) different food samples were collected from each street food vending location, particularly Rice, soup, and beans. Making a total of thirty (30) food samples. The food samples were collected with the dishing spoons used by the food vendors, packaged into sterile containers and were immediately transported to the laboratory for microbiological analysis.

Viable colony count and Isolation of Microbes

10g of each food sample was weighed and ground in a sterile mortar. 90ml of distilled water was poured into the mortar, the mixture was homogenized and used as stock followed by serial dilutions. Serial dilutions of up to 10^{-6} were made. 0.1ml of serial dilutions 10^{-3}, 10^{-4} and 10^{-5} were cultured on Nutrient Agar using the spread plate technique with a sterile glass rod. The petri dishes for the bacterial plate count were incubated at 37℃ for 24h. The number of colonies seen were counted using a colony counter and recorded as colony forming unit per gram (cfu/g).

Characterization and identification of microbes

Macroscopic examination and biochemical analysis of different isolates obtained from the different plates were accessed to identify the organism to the species level, using Bergey's manual of determinative bacteriology

Antibiotic Sensitivity Pattern of Pseudomonas aeroginosa

The antibiotic susceptibility test was performed to determine the levels of sensitivity and resistivity of some Gram negative drugs on *Pseudomonas aeroginosa* using the Mac Farland standard

Results

The result of thirty (30) food samples collected from ten (10) street food vending location mostly patronised by consumers in Ikpoba-Okha LGA is shown in the tables below. Table 1 shows the different food samples collected and analysed. Some of the food samples collected and analysed were beans (without oil) (26.7%), Jollof rice (20%), Egusi soup (13.3%)

Table 1. Food analysed

Food samples	Frequency	Percentage (%)
White rice and stew	3	10
Jollof rice	6	20
Fried rice	1	3.3
Beans (with oil)	2	6.7
Beans (without oil)	8	26.7
Egusi soup	4	13.3
Vegetable soup	3	10
Okro soup	3	10
Total	**30**	**100**

There was growth of *Pseudomonas aeroginosa* in many of the food samples analysed. The total viable count of *Pseudomonas aeroginosa* for SFL 5 had the highest count of 2.20 x 10^5 while the lowest count of 1.05 x 10^3 was found in SFL 1.

Table 2. Values for total viable count for *Pseudomonas aeroginosa*

Sample location	Mean TVC for bacteria (cfu/g)
SFL 1	$1.05 \pm 0.14 \times 10^3$
SFL 2	$1.90 \pm 0.27 \times 10^4$
SFL 3	0.00 ± 0.00
SFL 4	$0.38 \pm 0.01 \times 10^3$
SFL 5	$2.20 \pm 0.07 \times 10^5$
SFL 6	$0.33 \pm 0.12 \times 10^5$
SFL 7	$1.57 \pm 0.11 \times 10^4$
SFL 8	$1.50 \pm 0.20 \times 10^4$
SFL 9	$1.99 \pm 0.33 \times 10^3$
SFL 10	$1.70 \pm 0.23 \times 10^4$

TVC: Total viable counts; Mean ± SE (Standard error); cfu: colony forming units;

It was observed that *Pseudomonas aeroginosa* which had occurrence of 90% was isolated in food samples from all street food vending locations except SFL 3.

Table 3. Distribution of bacteria isolates in the different sample location

Bacteria found	Sample location									
	SFL 1	SFL 2	SFL 3	SFL 4	SFL 5	SFL 6	SFL 7	SFL 8	SFL 9	SFL 10
Pseudomonas aeroginosa	+	+	-	+	+	+	+	+	+	+

Table 4 shows the susceptibility of some selected strains of *Pseudomonas aeroginosa* isolated in food samples from the street food locations to different antimicrobial agents (gram negative antibiotics). The result showed that the isolate was sensitive to Ciprofloxacin (10µg), Gentamycin (10µg), Augmentin (30µg), Amikacin (30µg), Ceftazidime (30µg) and Norfloxacin (10µg) whereas, it was resistant to Cotrimoxazole (30µg), Ceftriazone (30µg), Tetracycline (30µg) and Erythromycin (10µg). It was observed that the isolate was more susceptible to Ciprofloxacin (10µg) and Norfloxacin (10µg).

Table 4. Susceptibility of *Pseudomonas aeroginosa* from food samples to antimicrobial agents

Antibiotics	(Concentration)	Susceptibility
Cotrimoxazole	(30µg)	R
Ceftriazone	(30µg)	R
Tetracycline	(30µg)	R
Ciprofloxacin	(10µg)	MS
Erythromycin	(10µg)	R
Gentamycin	(10µg)	S
Augmentin	(30µg)	S
Amikacin	(30µg)	S
Ceftazidime	(30µg)	S
Norfloxacin	(10 µg)	MS

S= Susceptibile, R= Resistant, MS= More Susceptible

Discussion

The results shows that there was *Pseudomonas aeroginosa* growth in many of the food samples analysed (Table 1) especially the soup samples. The growth of this organism which may be as a result contaminated vegetables (Kemajou *et al.,*2017) or meat used in cooking the foods (Nkanga and Uraih, 1981) and also as a result of poor hygiene practises of the food handlers, inadequate heating, secondary contamination via contact with contaminated equipment, utensils or surfaces and inappropriate processing (Gopal *et al*., 2015). It may also be due to microbial contamination of water used to wash equipment and utensils, large number of people crowding serving space or food sale point and long periods between preparation time and consumption time.

Food samples collected from SFL 5 in this study had the highest mean total viable count of 2.20 x 10^5 for *Pseudomonas aeroginosa* compared to the count from the other street food vending locations (Table 2). The isolate had high occurrence of 90% from all food samples collected from all the street food vending locations (Table 3). This is similar to the report Kemajou *et al.,* (2017), who reported counts of *Pseudomonas aeroginosa* isolated with other bacteria isolates from vegetables.

Pseudomonas aeroginosa can be found in nearly everywhere as long as there is enough water. Common habitats are moist soils and lakes as well as toilets, sinks, swimming pools, soap dishes and dishwashers (Zottola *et al.,* 1994). *Pseudomonas* biofilm formation is been reported to be problematic and a serious public health risk (Meliani and Bensoltane, 2015; Burmolle *et al.,* 2010). *Pseudomonas* biofilm can grow on abiotic surfaces of different equipment and processing surfaces in food industry food. It is also reported that when organisms like *pseudomonas* form biofilm, they became more resistant to the chemicals and antibiotics. Such a biofilm is a potential source of contamination of foods that may lead to spoilage, foodborne diseases and transmission of foodborne pathogens (Gunduz and Tuncel, 2006; Joseph *et al*., 2001).

Table 4 showed that *Pseudomonas aeroginosa* was sensitive to Ciprofloxacin (10µg), Gentamycin (10µg), Augmentin (30µg), Amikacin (30µg), Ceftazidime (30µg) and Norfloxacin (10µg). This is in accordance with the previous report of sensitivity of *Pseudomonas aeroginosa* to several antimicrobial agent (Kemajou *et al.,* 2017). Sensitivity of the isolate to Gentamycin, Ceftazidime and Amikacin were previously been reported by Igbinosa *et al*. (2011) and Odjadjare *et al*., (2012). It was also observed that the isolate was more susceptible to Ciprofloxacin (10µg) and Norfloxacin (10µg). This agrees with the reports of Tamil and Murugan (2011) and Bekele *et al*., (2015) who reported that *Pseudomonas aeroginosa* was more susceptible to the antimicrobial agents

It also showed that the isolate was resistant to Cotrimoxazole (30µg), Ceftriazone (30µg), Tetracycline (30µg) and Erythromycin (10µg). This agrees with the reports of Kemajou *et al.,* (2017) and Tamil and Murugan (2011) who resistance to same drugs. The observations suggests that the food samples from the street food vending locations were a considerable source of multidrug resistant strains of *Pseudomonas aeroginosa.*

Conclusions

The presence of *Pseudomonas aeroginosa* in food sold in street food vending locations which are highly patronised by consumers in Ikpoba-okha Local Government Area (LGA) of Edo State, is a cause for concern as it could lead to serious public health challenges of the consumers. Foodborne diseases induced by *Pseudomonas aeroginosa* can become

problematic especially in the case of multiple drug resistance. It is therefore recommended that Edo State government should enforce strict regulations and supervision on good hygiene practices such as the use of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) application in food production and processing, which will mitigate *Pseudomonas aeroginosa* contamination of food and foodborne diseases

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