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ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATES OBTAINED IN BREAD-PRODUCTION SALES CHAIN IN IFE CENTRAL LOCAL GOVERNMENT, OSUN STATE

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Abstract: The study was carried out to identify the bacterial isolates in the bread production- sales chain in Ife Central Local Government as well as to identify their antibiotic drug resistance. The present study was conducted on 52 bread samples. Predominant Gram positive micro-organisms found were 37(71.15%) Staphylococcus aureus, 2 (3.85%) Coagulase negative staphylococci, 2(3.85%) Streptococci spp. Two Enterobacter(3.85%) were the only gram negative microorganism found in the samples. Out of the Staphylococcus aureus isolated, 25 (67.57%) were resistant to all the antibiotics used, all the coagulase negative staphylococci were resistant to all the antibiotics except gentamicin, streptomycin and tetracycline. One Streptococcus was resistant to all antibiotics while enterobacter were resistant to cotrimoxazole, colistin and streptomycin.

Keywords: Antibiotic, Gram-positive microorganisms, Gram -negative microorganisms, drug resistance.

INTRODUCTION

Bread is a major source of food that can be consumed anywhere including homes, restaurants and hotels (Emeje et al, 2009). It is eaten all over the world by almost people of every culture and if we travel to the other side of the planet where we find a culture very different from our own, there will be its own version of bread (Eagle, 2002). It is one food item that anyone in a hurry to meet up with the day's activities can find to eat without spending much time preparing it. After rice, bread has become the second most widely consumed non-indigenous food in Nigeria (Shittu, 2007). It can be eaten with anything and at any time of the day. It also comes in different varieties in terms of size, flavour or manner of presentation and there seem to be one type that would suit anybody's preferences. The ease and accessibility of bread has made it to be in high demand (Omorogbe et al, 2010). Bread consists of the inner crumb and the outer crust. The crust has more dietary fibre and antioxidants, notably pronyl- lysine and the pronyl- lysine found in bread crust has been researched for its potential colorectal inhibitory properties (Paneerselvam, 2009). Flour is the main ingredient in making bread, other ingredients include salt, fat, leavening agents, milk, egg, sugar, spice such as raisings and the production process include weighing, mixing, milling, scaling and panning, proofing, baking, cooling, and packaging (WHO,1997).

Although bread is a prominent food for the world population, it can affect the health of the people when contaminated with pathogenic microorganisms. Basically, the surface of a fresh baked bread is free of viable microorganisms when hot; however, mould spores and bacteria from the air, improperly sanitized utensils, handlers, transporting equipments, wrapping materials can be sources of contamination to bread. Over 90% of bread contamination occurs during cooling, transporting, slicing and wrapping operation. (Ehavald, 2009) It was reported from Nigeria that freshly baked bread, after ten minutes had been contaminated with bacterial species which include *Bacillus cereus* and *Staphylococcus* spp. and after 48 and 96 hours *Staphylococcus cohnii* and

Bacillus firmus were isolated, respectively.(Ogundana,1986) Similarly, after 10 min mould such as *Aspergillus flavus, Aspergillus niger* and *Penicillium citrinum* were reported.

MATERIALS AND METHODS

Sample collection: Thirteen bakeries were randomly selected through balloting from the 65 registered bakeries in the study area. One bread each was picked from the cooling room, another sample was collected at the point at which the bread is being loaded into the vehicle. Another sample was collected at the point of delivery to the vendors while the last sample was collected at the point of sales. All these samples were collected using the microbiological standard for sample collection and the samples were subjected to microbiological analysis.

Media Preparation: Tryptone soy broth medium was weighed out and prepared according to the manufacturer's specification, with respect to the given instructions and directions; 20mls each were dispensed into macCartney bottles and sterilized by autoclaving at 121^{o}C for 15 minutes. After cooling, 5g of bread was inoculated into the medium and incubated for 24 hours. MacConkey and chocolate agar were also prepared according to the manufacturer´s instruction using the pour plate method and a loopful of the culture was streaked on the macConkey agar plates and duplicated on chocolate agar plates. The plates were incubated for 48 hours after which biochemical tests were carried out on the bacteria found on the plates. The biochemical tests included gram staining, catalase test, Sulphide indole motility (SIM) test, triple sugar ion (TSI) test, indole test and antibiotic sensitivity test.

Susceptibility of Isolates to Various Antibiotics

Antibiotic sensitivity tests were carried out on all isolates using paper (New Man England) disc diffusion technique. A total of 8 antibiotics were tested for gram positive organisms and 9 antibiotics for gram negative organisms. 0.2ml of 12h peptone water culture of test organism was used to inoculate each organism on a dry sterile nutrient agar plate. The resistant profiles of bacteria isolated from bread were determined by standard methods. The antibiotic sensitivity disc for gram positive cocci bacteria contained Ampicillin(10 microgram), chloramphenicol(10 microgram), cloxacillin(5 microgram),erythromycin(5 microgram),gentamicin(10 microgram), penicillin(11.U.), streptomycin(10 microgram), and tetracycline(10 microgram) while the disc for gram negative organisms contained amoxicillin(30 microgram), cotrimoxazole(25 microgram), gentamicin(10 microgram), nalidixic acid(30 microgram), nitofurantoin(300 microgram), colistin(10 microgram), streptomycin(10 microgram), tetracycline(10 microgram) and ciprofloxacin(30 microgram). Nutrient agar was the media used. Each of the isolates was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry for about 15 to 30 minutes. The antibiotic discs were placed on agar using sterile forceps. The plates with the antibiotic discs were then incubated at 37^{o}C for 24 hours to observe the zones of growth inhibition produced by the antibiotics and recorded immediately.

RESULTS:

Table1: bacteria isolated at the different point in bread distribution chain

Sample space	***S. Aureus***	***Streptococci spp.***	***Bacillus spp.***	**Coagulase negative *S. Aureus***	**No growth**
Cooling	10	1	0	0	2
Loading to the vehicle	10	0	0	1	2

Delivery to vendors	10	0	0	0	3
From vendors	8	2	1	1	1
Total	**38**	**3**	**1**	**2**	**8**

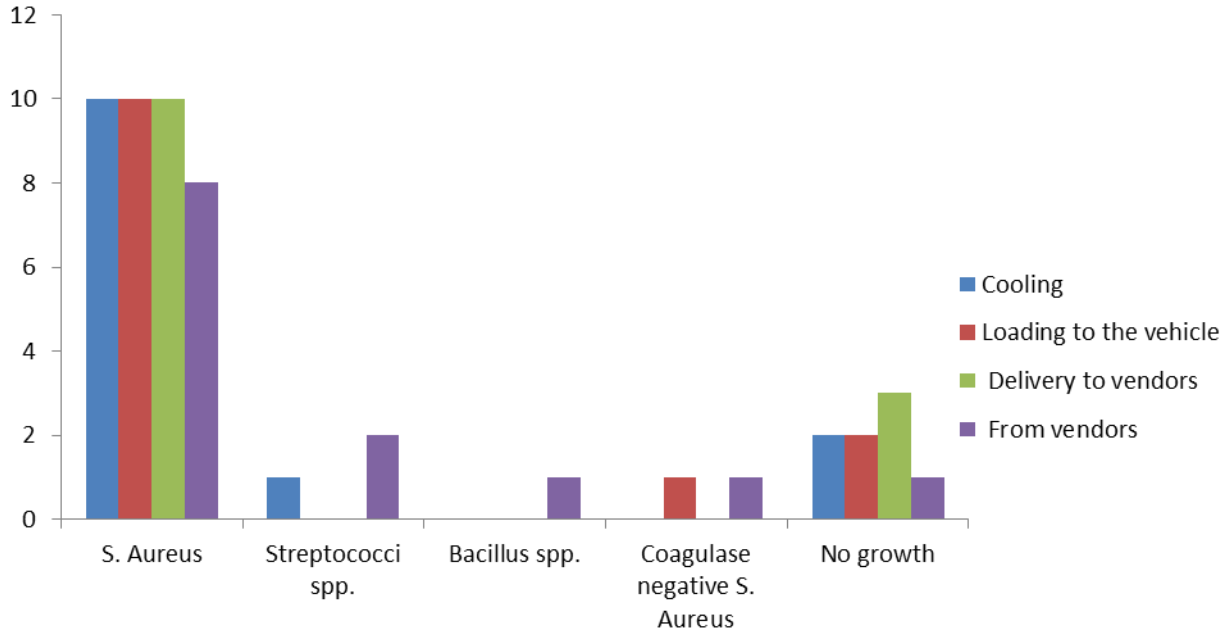

Figure above comparing the point of contamination in bread distribution chain

Table 2: Sentivity of isolates to diferent antibiotics

isolates	**Resistance** (Resistant to all antibiotics used)	**Highly sentitive** (sensitive to all antibiotics used)	**Fairly sentitive** (Sensitive to all antibiotics used)
S. Aureus	25	0	15
Coagulase negative S. Aureus	0	0	2
Streptococcus spp.	1	0	1

DISCUSSISON

The percentage occurrence of staphylococcus aureus in this study was 71.15%. This result is higher than some previously reported detection such as 11.76% (Odewade et al, 2018), 5.5% (Esfarjani et al, 2018), 20% reported by Ojima *et al*. (Kennedy, 2005), or 27.3% (Otu-Bassey *et al*. 2002), 68.9% (Shiferaw et al, 2018). *Staphylococcus aureus,* is a common inhabitant of the body which occupy up to 50% of the human nose, throat, and skin (Arbutnoth, 1990).

In the present study, the antibiotic resistance patterns of the isolates revealed that more of the Staphylococcus aureus were resistant to penicillin and this is similar to a study where all of *S. aureus* isolates were resistant to three Methicillin group antibiotics including Methicillin, Oxacillin and Penicillin G. (Shiferaw, 2018)
Also, slightly more than half of the Staphylococcus aureus (51.92%) were resistant to Erythromycin and this is higher than that of a study in which 40.3% of the staph aureus were resistant to Erythromycin. (Shiferaw, 2018) This is also in agreement with Alexandra et al. (Alexandra, 2011) who reported that, 100% of the isolates were resistant to most Methicillin group antibiotics, also this is higher than that of the study carried out by Temilade (Temilade, 2009) isolates in which out of 106 isolates of *S. aureus*, 40.6% were resistant to erythromycin, 63.2% to penicillin G, and 20.7% resistant to oxacillin. This could be that the isolates *S.aureus* carry mecA gene that encodes a variant Penicillin binding protein (PBP2a or due to the production of penicillinase enzyme that hydrolyzed the beta-lactam ring of penicillin derivatives antibiotics (Lowy, 2003).

The alarmingly emerging of Methicillin Resistant Staphylococcus aureus (MRSA) could be due to integration of genetic mobile elements such as plasmids, transposons, and insertion sequence in case of inappropriate or uncontrolled use of antibiotics (Deleo, 2009; Szweda,2012). Therefore, it is necessary to pay more attention to food hygiene practices to reduce or eliminate the risk from resistance to antibiotics and pathogenic bacteria originating from food (Van, 2007).

However, enterobacter was not resistant to ciprofloxacin in contrast to the study carried out on cancer patients in Egypt where there was evident resistance to ciprofloxacin by *E. coli*, *Klebsiella* and *Enterobacter* species. (Ramadan, 2011) The return to the pre-antibiotic era has become a reality in many parts of the world. Multi-drug resistant (MDR) microorganisms were recently named as the 'ESKAPE' pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species), indicating their 'escape' from the effects of antibacterial agents or the non-existence of newer active antibiotics (Giamarellou, 2010). The lack of alternative agents that are active against gram-negative bacteria necessitates the use of measure for controlling emergence of resistance in bacterial strains.
Previously, multiple resistance to antibiotics by microorganisms were found mostly in hospitals where antimicrobial agents are used most frequently but resistance is found in refrigerated processed food materials as reported by a study, (Odewade et al, 2018) which agreed with the result obtained in this study.

The clinical management of a number of food-borne infections have been complicated by antimicrobial resistant bacteria (Bryan, 1992). More than 90% of bread contamination occurs during cooling, transporting, slicing and wrapping processes. (Ehavald and Estonia, 2009)

CONCLUSION

From the results and observations obtained from this study, it can be concluded that bread can also become vehicle of food -borne infections. These results should be very alarming to the public health authorities responsible for setting and implementing the antibiotic policies. The antibiotic policy must be reviewed and special measures should be taken to reduce the spread of antibiotic resistance among bacterial infections. A careful monitoring of anti-microbial use is required to identify the situation in which prescription patterns are contributing to the development of resistance. The lack of any new compounds in the near future indicates that there is need for constant monitoring at national, regional level as these surveillance efforts are essential to provide clinicians with information for choosing empirical treatment regiments and implement strict antibiotic prescribing policies and infection control guidelines. Screening for Extended Spectrum Beta-Lactamase (ESBL) production as a routine procedure in clinical laboratories gives valuable information to the clinician in appropriate selection of antibiotics. Moreover, bacterial strains resistant to most classes of antibiotics will continue to arise unless the inappropriate use of these drugs is curtailed

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APPENDIX

Key: AMP=Ampicillin, CHL= Chloramphenicol, CLO= Cloxacillin, ERY= Erythromycin, GEN= Gentamicin, PEN= Penicillin, STR= Streptomycin, TET= Tetracycline, AMX= Amoxicillin, COT= Cotrimoxazole, GEN= Gentamicin, NAL= Nalidixic oxide, NIT= Nitrofurantoin, COL= Colistin, CFL= Ciprofloxacin. S= Sample

Table below showing the characterization of bacteria isolates and Antibiotic susceptibility

Sample (S)	**Gram Reaction**	**Catalase**	**Coagulase**	**Organism**	**Sensitivity**
S1 Cooling	Positive cocci	Positive	Positive	Staph aureus	Resistant to all
S1 Loading to the vehicle	Positive cocci	Positive	Positive	Staph aureus	Resistant to all
S1 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S1 From vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S2 Cooling	Positive cocci	positive	positive	Staph aureus	Resistant to all
S2 Loading to the vehicle	Positive cocci	positive	positive	Staph aureus	Resistant to all
S2 Delivery to vendors	Positive Bacilli				
S2 From vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to ERY
S3 Cooling	No growth				
S3 Loading to the vehicle	No growth				
S3 Delivery to vendors	No growth				
S3 From vendors	No growth				
S4 Cooling	Positive cocci	positive	positive	Staph aureus	Sensitive to GEN,ERY,TET,STR
S4 Loading to vehicle	Positive cocci	positive	positive	Staph aureus	Resistant to all
S4 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to GEN,ERY,STR,TET
S4 From vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to GEN,ERY
S5 Cooling	Positive cocci	positive	positive	Staph aureus	Sensitive to TET
S5 Loading to vehicle	Positive cocci	positive	positive	Staph aureus	Sensitive to GEN,STR,TET
S5 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S6 Cooling	Positive cocci	positive	positive	Staph aureus	Sensitive to STR,GEN
S6 Loading to the vehicle	Positive cocci	positive	positive	Staph aureus	Sensitive to TET
S6 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all

S6 From vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to GEN,TET,CHL
S7 Cooling	Positive cocci	positive	positive	Staph aureus	Sensitive to ERY
S7 Loading to vehicle	Positive cocci	positive	positive	Staph aureus	Resistant to all
S7 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S7 From vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to TET,GEN,ERY
S8 Cooling	Positive cocci	positive	positive	Staph aureus	Sensitive to ERY
S8 Loading to vehicle	Positive cocci	Positive	positive	Staph aureus	Resistant to all
S8 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S8 From vendors	Positive cocci	positive	negative	Coagulase negative staph	Sensitive to GEN,STR,TET
S9 Cooling	positive cocci	positive	positive	Staph aureus	Resistant to all
S9 Loading to vehicle	Positive cocci	positive	positive	Staph aureus	Resistant to all
S9 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S9 From vendors	Positive cocci	positive	positive	Staph aureus	Sensitive to STR,TET,ERY,GEN
S10 Cooling	Positive cocci	positive	positive	Staph aureus	Resistant to all
S10 Loading to vehicle	Positive cocci	positive	positive	Staph aureus	Resistant to all
S10 Delivery to vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S10 From vendors	Positive cocci	positive	positive	Staph aureus	Resistant to all
S11 Cooling	Positive cocci	positive	Positive	Staph aureus	Resistant to all
S11 Loading to vehicle	Positive cocci	positive	Negative	Coagulase negative Staph	Sensitive to GEN,STR,TET
S11Delivery to vendors	Positive cocci	positive	Positive	Staph aureus	Resistant to all

S11 From vendors	Positive cocci	negative	Negative	Streptococus spp	Resistant to all
S12 Cooling	No growth				
S12 Loading to vehicle	No growth				
S12 Delivery to vendors	No growth				
S12 From vendors	Positive cocci	negative	Negative	Streptococcus spp	Sensitive to ERY,CHL
S13 Cooling	No growth				
S13 Loading to vehicle	Positive cocci	positive	Positive	Staph aureus	Resistant to all
S13 Delivery to vendors	Positive cocci	positive	Positive	Staph aureus	Resistant to all
S13 From vendors	Positive bacilli				

Test Sample	S5 From vendors	S9 Cooling
Gram Reaction	Negative bacilli	Negative bacilli
Indole	Negative	Negative
Citrate	Negative	Positive
Urease	Negative	Negative
Motility	Positive	Positive
Lactose	Positive	Positive
Sucrose	Positive	Positive
Glucose	Positive	Positive

Gas	Negative	Positive
Hydrogen Sulphide	Negative	Negative
Organism	Enterobacter spp	Enterobacter spp
Sensitivity	Sensitive to CFL,TET,NIT,NAL	Sensitive to GEN,NAL,CFL,TET,AMX,NIT