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Physiological Studies and Antibiotic Resistance Profile of Bacterial Pathogen Isolated from Some Nigerian Fast Food

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ABSTRACT

Most food handlers in fast food centers have no knowledge of food safety practices and of prevention of food-borne diseases which has resulted in the transmission of food-borne pathogens to the people consuming such food. Therefore, this study was aimed at evaluating the level of bacterial contamination in some fast food in Ogbomoso, Nigeria and to determine the antibiotic susceptibility and physiological profile of the bacterial contaminants. Bacterial pathogens were isolated from fast food vended in Ogbomoso, Oyo state, Nigeria. The isolates were characterized and identified as *Enterobacter aerogenes, Aeromonas hydrophila, Pseudomonas putida, Escherichia coli, Proteus vulgaris, Micrococcus luteus, Bacillus cereus, Pseudomonas chlororaphis* and *Bacillus subtilis*. The total bacteria colony count ranged from 2.4×10^4-4.2×10^6. The antibiotic susceptibility profile of the isolates was determined and 75% sensitivity to the clinically relevant antibiotic disc was noted while 25% resistance was found. The effect of physiological parameters including temperature, pH and sodium chloride concentration on the growth rate of isolates was evaluated. As temperature of incubation increased from 50-80°C, the rate of growth of all the isolate decreased and as the pH of the growth medium increased from 3-9, the rate of growth of all the isolates also increased. As the concentration of sodium chloride increased from 2-5%, the rate of growth of isolates also reduced. The results of this study showed that most of the fast food samples examined did not meet bacteriological quality standards. Hence, it is recommended that a regular monitoring of fast food should be carried out by putting in place appropriate agency.

Key words: Food quality, growth rate, pH, temperature, sodium chloride and bacterial contaminant

INTRODUCTION

Any food with low preparation time and that can be served very quickly is referred to as fast food (Jakle and Sculle, 1999) In addition, fast food can be said to refer to food sold in a restaurant or store with low quality preparation and served to the customer in a packaged form for take-out or take away (Talwar and Jennifer, 2003). Furthermore, the term also connotes speed in both food preparation and customer service, as well as in customer eating habit. Fast foods restaurant industry is traditionally designated as 'quick service' (Bowman *et al.*, 1998).

Among fast food restaurant and any other restaurant where consumer's health is sacrificed because of speed and profit; food contamination is a serious problem (Ross, 2008). However, in

abroad sense, food contamination might be due to presence of micro organism like *E. coli*, *Pseudomonas* sp., *Micrococcus* sp. etc. in food such as undercooked food or raw food stuff which in one way or the other serve as a host for such microbes. A report from Nevada, Arizona and California showed that there was a great out-break of *E. coli* which led to people suffering from bloody diarrhoea and renal problems as a result of contact of manure from unhealthy cattle with the meat from the slaughter house. Food contamination might be as a result of teenager and unskilled personnel (likely adult) employed in the business as crew, with low pay which in one way or the other handle foods in an unhealthy manner (Cardia study).

Furthermore, food poisoning refers to illness caused by all type of food borne micro-organisms. There is difference between food poisoning and food infection, although, the symptoms are similar. Food poisoning is caused by eating food that contains poison or toxin produced by bacteria in the food. The toxin producing bacteria may be killed, but the toxin they produced causes illness or digestive upset (Anderson and Baird-Parker, 2000). While, food infection is caused by eating food that contains certain type of live bacteria and once the food is consumed, the bacteria cells begin to grow and illness can result (Emerson and Robert, 2003).

Fast food companies has been blamed for the poor quality and unhealthy food they produce for people in that they are always after selling their product not showing the details about what is in it. The companies hide all kind of information, like what they put in their food, how it is package or produced and even to the major health risk it cause to the consumers (Fakunaga *et al.*, 2002). As work on fast foods, their health effects on consumption as well as the extent of fast food contamination keep growing globally, the fast food industry as decided to adjust in other to avoid losing customers. Nevertheless, getting health and nutritional information about the food to be consumed is a hard thing to do on the part of fast food service provider. Therefore the aims of this study were to isolate and identify bacteria from some Nigeria fast food, to evaluate the effect of some physiological parameters like pH, temperature and different concentration of sodium chloride on the growth of isolates, also to evaluate the resistance of the isolates against clinically relevant antibiotics.

MATERIALS AND METHODS

Collection of samples and location: The study was carried out in science laboratory Technology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria between, March 2009 and January 2010. The fast food samples were collected in Ogbomoso, Oyo state. The samples included meat pie, fish pie, egg buns, gala, jam-doughnut and suya. All samples were bought from fast-food restaurants in Ogbomosho and taken to the laboratory for analysis.

Culture media: The culture media used include nutrient agar, Eosin methylene blue agar, MacConkey agar and Salmonella Shigella agar. The medium were prepared according to the manufacture specification. These medium were sterilized in an autoclave at 121°C for 15 min.

Total colony count: One gram of each sample was dissolved in sterile de-ionized water and serially diluted. One milliliter of appropriate dilutions was seeded on plate count agar using spread plate method and the medium was then incubated at 37°C for 24 h. The plate count agar was examined and colonies present were counted and recorded after incubation at 37°C for 24 h, to get the total colony count in CFU g^{-1}.

Isolation of microorganisms: One gram of each fast food sample was serially diluted, 1 m of an appropriate dilution was inoculated on the MacConkey, Nutrient agar, Salmonella shigella and the plate was incubated for 24 h at 37°C. After 24 h sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared nutrient agar then incubate for 24 h at 37°C in order to get pure cultures. Pure cultures were then stored in a refrigerator at 4°C. The routine laboratory method of Cruickshank *et al.* (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Antibiotic sensitivity test: Mueller-Hinton agar was evenly seeded throughout the plate with the isolate which had been previously diluted at a standard concentration (approximately $1\text{-}2\times10^8$ colony forming units per mL). Commercially prepared disks, each of which was pre-impregnated with a standard concentration of a particular antibiotic, were lightly pressed onto the agar surface; the plates were incubated for 24 h at 37°C. The antibiotics used included ampicillin, gentamicin, nitrofurantion, ciprofloxacin, tetracycline, norfloxacin, amoxycillin, ofloxacin, chloramphenicol, cefuroxime, erythromycin, co-trimoxazole, cephalexin, ceftriaxone, ampicillin/cloxacillin, clindamycin and augmentin. After an overnight incubation, the bacterial growth around each disc was observed.

Physiological study of isolates
Growth of isolates at different temperatures ranges: Nutrient broth was prepared and dispensed into series of capped bottles and sterilized. It was allowed to cool and the test organisms inoculated into it, then incubated at different temperature ranges (50, 60, 70 and 80°C) for 24 h after which Cecil 2031 (automatic) spectrophotometer was used to detect increase or decrease in turbidity. Un-inoculated tubes served as control. This test was done to determine best temperature that favours growth and metabolism as indicated by increased turbidity (Gibson and Abdel-Malek, 1945).

Growth of isolates at different pH ranges: Nutrient broth was prepared and the pH was adjusted using 0.1 phosphate buffer of different pH to adjust the pH of the broth to 3.0, 5.0, 7.0, 9.0, it was then dispensed into screw-capped bottles and then sterilized in the autoclave at 121°C for 15 min. After cooling, the various test isolates were inoculated into it and incubated at 30°C for 24 h. Growth was detected by increased turbidity using Cecil 2031(automatic) spectrophotometer. Un-inoculated tube served as control. This test was done to detect the best pH that favours growth and metabolism as indicated by the increased turbidity (Schillinger and Lucke, 1989).

Growth of isolates at different concentration of sodium chloride: Nutrient broth containing 2, 3, 4 and 5% (w/v) NaCl was prepared and sterilized at 121°C for 15 min. Ten milliliter of the broth was then dispensed into sterile screw-capped vials aseptically. After cooling the tubes were inoculated with the test organisms and incubated for 24 h at 30°C. Increased turbidity of the medium was recorded as positive for growth while a negative result shows no turbidity. Un-inoculated tubes served as control (Schillinger and Lucke, 1987).

RESULTS
A total of nine organisms were isolated from different fast food samples vended in Ogbomoso, Oyo state, Nigeria. The isolates were initially differentiated on the basis of the cultural and

morphological studies after which they were subjected to various biochemical tests such as oxidase test, oxidase test Gram staining, Gelatin hydrolysis, catalase test etc. and were identified with the aid of Bergey's Manual of Systematic Bacteriology. The isolates were identified to be *Enterobacter aerogenes, Aeromonas hydrophila, Pseudomonas putida, Escherichia coli, Proteus vulgaris, Micrococcus luteus, Bacillus cereus, Pseudomonas chlororaphis* and *Bacillus subtilis.*

The values for total colony count for bacterial isolates is shown in Table 1, suya showed the highest colony count of 4.2×10^6 and gala showed the lowest total colony count of 2.4×10^4.

The distribution of the four bacteria species present in all samples is shown in Table 2. *Bacillus cereus, E. coli* and *Pseudomonas chlororaphis* were present in suya while *Bacillus subtilis, Micrococcus luteus* and *Aeromonas hydrophila* were present in meat pie.

Antibiotic susceptibility test was also carried out and it was done using agar diffusion method. Isolates were resistant to Amoxycillin (AM) except *Enterobacter aerogenes*, All the organisms were sensitive to Gentamicin (GN) except *Proteus vulgaris* and *Bacillus subtilis*. All the organisms were resistant to Nitrofurantion (N) except *E. coli* and *Enterobacter aerogenes*. Nearly all organisms were sensitive to Ciprofloxacin (CIP) with the exception of *Pseudomonas putida* and *Pseudomonas chlororaphis*. Most of organisms were resistant to Tetracycline (TE) except *E. coli, Pseudomonas putida* and *Enterobacter aerogenes*. Five of organisms were resistant while the remaining four were sensitive to Norfloxacin (NB). All organisms were resistant to Amoxycillin (AX) except *Enterobacter aerogenes*. All organisms were sensitive to Ofloxacin (OF) except *Pseudomonas chlororaphis* and *Bacillus subtilis*. Most of organisms were resistant to Chloramphenicol (C) except *E. coli, Pseudomonas putida* and *Enterobacter aerogenes*. All organisms were resistant to Cefuroxime (CF) except *Enterobacter aerogenes*. All organisms were resistant to Erythromycin (E) except *Micrococcus luteus* and *Bacillus cereus*. All organisms were resistant to Cephalexin (CX), Co-trimoxazole (CO), Ceftriaxone (FX), Ampicillin/Cloxacillin (AP) and Augmentin. Organisms were resistance to Clindamycin (CD) except *Bacillus cereus* (Table 3).

The growth rate of isolates at different temperature was tested and it was observed that as the temperature increased the rate of growth of all the organisms decreased. As the temperature

Table 1: Total colony count for isolates

Sample code	Total colony count for bacteria (CFU g^{-1})
Gala	2.4×10^4
Doughnut	2.8×10^4
Suya	4.2×10^6
Meat pie	4.0×10^6
Egg buns	3.0×10^4

Table 2: Distribution of bacteria isolates in the different samples

Isolates	Suya	Doughnut	Gala	Meat pie	Egg buns
Bacillus cereus	+	-	-	-	-
Escherichia coli	+	-	-	-	-
Enterobacter aerogenes	+	-	-	-	-
Pseudomonas chlororaphis	-	+	-	-	-
Proteus vulgaris	-	-	+	-	-
Bacillus subtilis	-	-	-	+	-
Micrococcus luteus	-	-	-	+	-
Aeromonas hydrophila	-	-	-	+	-
Pseudomonas putida	-	-	-	-	+

Table 3: Antibiotic susceptibility profile of the isolated bacteria

Isolates	Antibiotics																
	AM	GN	N	CIP	TE	NB	AX	OF	C	CF	E	CD	CO	CX	FX	AP	AU
Enterobacter aerogenes	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R
Aeromonas hydrophila	R	S	R	S	R	S	R	S	R	R	R	R	R	R	R	R	R
Pseudomonas putida	R	S	R	R	S	R	R	S	S	R	R	R	R	R	R	R	R
Pseudomonas chlororaphis	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Escherichia coli	R	S	S	S	S	S	R	S	S	R	R	R	R	R	R	R	R
Proteus vulgaris	R	R	R	S	R	S	R	S	R	R	R	R	R	R	R	R	R
Micrococcus luteus	R	S	R	S	R	R	R	S	R	R	S	R	R	R	R	R	R
Bacillus cereus	R	S	R	S	R	R	R	S	R	R	S	S	R	R	R	R	R
Bacillus subtilis	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R

R: Resistant, S: Sensitive, Ampicillin: AM 10 mcg, Gentamicin: GN 10 mcg, Nitrofurantion: N 100 mcg, Ciprofloxacin: CIP 5 mcg, Tetracycline: TE 50 mcg, Norfloxacin: NB 10 mcg, Amoxycillin: AX 20 mcg, Ofloxacin: OF 5 mcg, Chloramphenicol: C 10 mcg, Cefuroxime: CF 30 mcg, Erythromycin: E 10 mcg, Co-trimoxazole: CO 50 mcg, Cephalexin: CX 30 mcg, Ceftriaxone: FX 30 mcg, Ampicillin/Cloxacillin: AP 30 mcg, Clindamycin: CD 10 mcg, Augmentin: AU 30 mcg
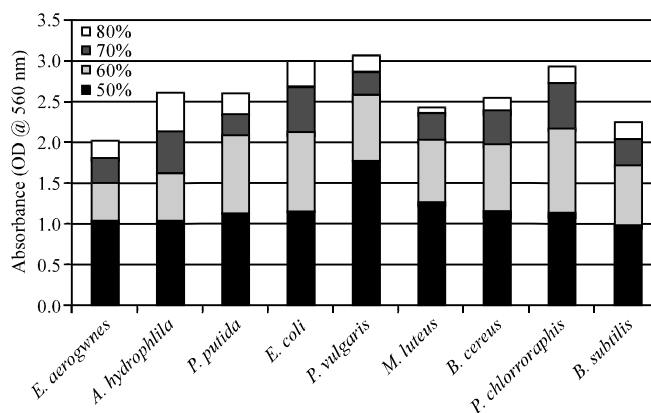

Fig. 1: Rate of growth of isolates at different temperature ranges

increased from 50-80°C, the optical density reading for *Enterobacter aerogenes* decreased from 1.030-0.187, *Aeromonas hydrophila* decreased from 1.046-0.462 and *Pseudomonas putida* from 1.122-0.240 (Fig. 1).

The survival of isolates in different pH ranges was tested and it was observed that the rate of growth increased as the pH increased. As the pH increases from 3-9, the optical density reading for *Enterobacter aerogenes* increased from 0.100-0.980. *Aeromonas hydrophila* increased from 0.901-1.045 and *Pseudomonas putida* from 0.065-1.240 (Fig. 2).

The rate of growth of the isolates was also observed in different concentration of sodium chloride. It was found that as the concentration of sodium chloride increased the rate of growth of isolates decreased. As the sodium chloride from 2-5% the optical density reading for *Enterobacter aerogenes* decreased from 1.566-0.740, *Aeromonas hydrophila* decreased from 1.196-0.703 and *Pseudomonas putida* decrease from 1.511-0.989 (Fig. 3).
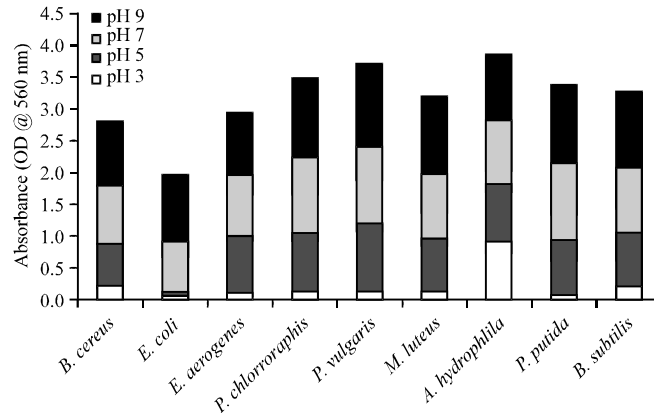
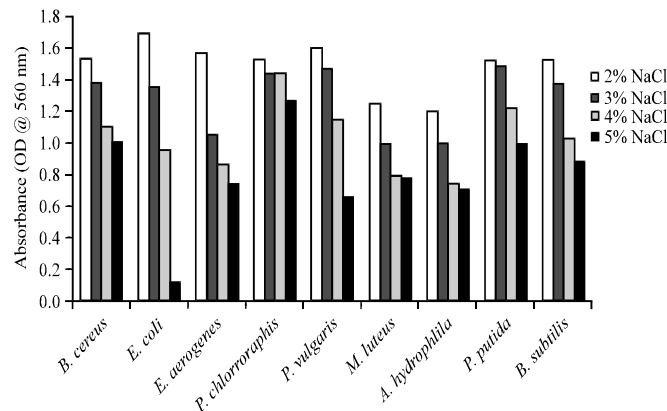Fig. 2: Rate of growth of isolates at different pH ranges (OD at 560 nm)

Fig. 3: Rate of growth of isolate in different concentration of sodium chloride (NaCl)

DISCUSSION

This study revealed the presence of the some bacterial pathogen in some Nigerian fast food evaluated; the bacteria included *Enterobacter aerogenes, Aeromonas hydrophila, Pseudomonas putida, E. coli, Proteus vulgaris, Micrococcus luteus, Bacillus cereus, Pseudomonas chlororaphis* and *Bacillus subtilis*. This is in agreement with the previous work of (Oladipo and Adejumobi, 2010) who reported the presence of *Bacillus licheniformis, Aeromonas hydrophila, Enterobacter aerogenes, Bacillus cereus, Proteus mirabilis, Pseudomonas putida, Proteus vulgaris, Pseudomonas chlororaphi* and *Proteus morganii* in some street vended cooked foods. Wogu *et al.* (2011) also recovered *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia* from ready-to-eat rice. The presence of the isolated bacteria pathogens is of great risk to consumer's health and should be of great concern.

Suya showed the highest colony count of 4.2×10^6 and *Bacillus cereus, E. coli, Pseudomonas chlororaphis* were isolated from this sample while *Proteus vulgaris* isolated from gala showed the lowest total colony count of 2.4×10^4. This is in agreement with the work of Wogu *et al.* (2011) who reported total bacteria colony count ranged from 2.0×10^4-1.2×10^6. International Commission on Microbiological Specifications for Foods stated that the presence of *E. coli* in

ready-to-eat foods is undesirable because it indicates poor hygienic conditions which have lead to contamination or inadequate heat treatment. The family *Enterobacteriaceae* includes many bacteria that are found in the human or animal intestinal tract, including human pathogens such as *Salmonella* and *Shigella. Enterobacteriaceae* are useful indicators of hygiene and of post-processing contamination of heat processed foods. Their presence in high numbers ($>10^4\,\text{g}^{-1}$) in ready-to-eat foods indicates that an unacceptable level of contamination has occurred or there has been under-processing (e.g., inadequate cooking). Furthermore, the presence of *Pseudomonas* sp. which is a pathogenic organism renowned for its high resistance to antibiotics, is a cause for concern (Oladipo *et al.*, 2009). An unsatisfactory level of *B. cereus* in cooked foods generally occurs as a result of inadequate temperature control above 60°C or at or below 5°C to prevent growth, or held outside this temperature range for a limited time. *Bacillus* sp. produces enterotoxin which could be deadly when ingested into the body (Oladipo *et al.*, 2009). The detection of high levels ($>10^8\,\text{CFU g}^{-1}$) of *B. cereus* should result in an investigation of the food handling controls used by the food business.

Sunde (2005) reported that high rates to antibiotics resistance of bacteria may possibly resulted from inappropriate or uncontrolled use of antibiotics in the farming practices. Moreover, Abbar and Kaddar (1991) reported that bacteria becomes resistant to antimicrobial agents through a variety of mechanisms which are; production of enzymes which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of antibiotics and development of metabolic pathways by bacteria which enable the site of an antimicrobial action to be by passed. Based on antibiotic susceptibility result, the micro-organisms were 75% sensitive to clinically relevant antibiotics while 25% was found resistant to the antibiotic used. The level of resistance and sensitivity of these bacteria to clinically relevant antibiotics differs, 75% was found sensitive to the antibiotics mentioned in the result and 25% was resistant to the antibiotics. 53.85% resistance and 46.15% sensitivity to antibiotics was reported by Oladipo and Adejumobi (2010).

Result obtained from the survival of isolates at different pH ranges showed that in acidic medium, the micro organisms growth rate were reduced but the growth rate increased as the pH of the growth medium changed to basic. This implies that acidic medium reduced the isolates growth greatly while the isolates flourished well in basic medium. Evaluation of survival rate of isolates at different temperature showed that as the temperature increased, the rate of growth of all the organisms decreased. Most of the organisms isolated have been reported to adapt well to environmental stress and as a result, it is always a challenge to eliminate them from the environment. This fact is reflected in the physiological study of the isolates. Increasing physiological parameters such as temperature and sodium chloride did not eliminate but rather reduced the rate of growth of the vended food isolates.

Moreover, it is agreed upon that most fast food are not hygienic for consumption due to poor preparation to quicken the readiness, incompetence of the workers which leads to contamination of most food, unhygienic condition of the materials and raw foods used in preparing such food despite clean packaging container, all these and more called for a strong monitoring from the relevant agency. The alarming rate of pathogenic bacteria growth in fast foods must be reduced by putting in place appropriate agency. To avoid future pathogenic outbreaks the quality of the fast food, hygienic condition as well as the competency of the workers in the restaurant should be regularly monitored.

CONCLUSION

Finally, most of the fast food samples evaluated did not meet microbiological national standards and many kind of pathogenic bacteria were found which may be a source of foodborne disease.

Most of the organisms isolated survived different environmental stress they were subjected to, which implies that it may be a challenge to eliminate them from the environment. Hence, a food hygiene practice is paramount in order to reduce or eliminate the risk from foodborne pathogens. Also, for fast food service provider's strict implementation of food sanitation code and license should be put in place so that consumer's health would not be at risk.

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