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Antibiotic Susceptibility Pattern of Bacteria Isolated from some Packaged Fruit Juice Marketed in Bauchi Metropolis, Nigeria

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Abstract:- Eight (8) samples of packaged fruit juice which include mango, peach, orange, strawberry, apple, apple/mango, pineapple and orange juice were analyzed for the presence bacterial pathogens using standard microbiological techniques. These fruit juices were purchased from retail shops in Bauchi metropolis, Nigeria. Total bacterial cell count of some of the packaged fruit juice sample ranges from 3.8 x 10^4 cfu/ml (for mango juice), 1.1 x 10^5cfu/ml (for peach juice), 2.5 x 10^4 (for orange and orange 2 juice), 2.9 x 10^4 (for strawberry juice) 2.0 x 10^4 (for apple juice) and 5.0 x 10^4 for (apple/mango fruit juice. Bacteria isolates obtained from the packed fruit juice includes; Staphylococcus aureus (30%), Pseudomonas aeruginosa (30%), Klebsiella spp (20%) and Escherichia coli (20%). Antibiotic susceptibility testing of the isolates showed that many of the bacteria had an intermediate, resistant and sensitive reaction to most of the antibiotics tested. The bacterial species isolated were 80% sensitive to ciprofloxacin (5µg), 70% sensitive to amoxicillin (25µg) while it showed a 70% resistance pattern to tetracycline(30µg), 60% resistance to ceftazidime(30µg). Some showed intermediate sensitivity to gentamicin (10µg). Due to the number and types of bacteria isolated from the different packaged fruit juices, it can be concluded that bacteria are present within fruits and the materials used for the production of the juice, as well as poor sanitary condition, raw material contaminations, lack of both proper heat sterilization and adequate quality control during processing of fruit juice could be the reasons for this contamination.

Keywords:- Antibiotic Susceptibility, Bacterial Count, Bauchi, Fruit Juice.

I. INTRODUCTION

Fruits are a part of our daily consumption worldwide and in everyone's diet chart it is always included as a whole fruit, beverage, or soft drink etc. Fruit juices are important sources of antioxidant, vitamin, and minerals which are essential for human being and may reduce the risk of heart disease, cancer, and diabetes. Fruit juice contain essential nutrient that serves as a medium for the growth of acid-tolerant bacteria, yeasts, and molds. In recent years, there has been emphasis on the need for chemically and microbiologically safe food as part of consumer awareness (Aneja, 2014).

The existence of microorganism like bacteria, yeast and mould in fruit juice are responsible for fermentation, food spoilage and foodborne illness (Yeh *et al.*, 2004, Essien *et al.*, 2011). These contaminations may occur due to simple packaging operation in the absence of aseptic condition (Juhania Kova *et al.*, 2003).

The most diversified microorganisms found in processed fruit juice causing its spoilage are bacterial pathogens. Lactic acid bacteria *Acetobacter* and *Acetomcens* found on fruit surfaces comprise the most frequent spoilers of fruit juice because they exist on the surface of plant and fruits growing at the expense of secreted plant material (Frazier *et al.*, 1998). Most fruit juice have high acidic and sugar content which favours the growth yeast (Hoover, 1997). Mould are generally considered to be the least important group of microorganisms causing spoilage in fruit juice because of their inability to grow in the absence of air (Panoh, 1991) though with few expections such as *Penicillium* and *Aspergillus* spore forming (Frazier *et al.*, 1998).

Some food borne illness have been reportedly associated with consumption of fruit juice in several place (Muinde *et al.*, 2005). Food borne disease affects the gastrointestinal tract (GIT) and can be transmitted through consumption of contaminated food or drink. Report suggests that some juice may be potential source of bacterial pathogens like *E. coli* 0157:H7, *Staphylococcus aureus*, *Salmonella spp.* and *Shigella spp* (Barro, *et al.*, 2006). The aim of the study was to determine the antibiotic susceptibility pattern of bacteria isolated from some packaged fruit juice sold in Bauchi metropolis.

II. MATERIALS AND METHODS

A. *Sample Collection*

Eight (8) sample of packaged orange, apple, strawberry and mixed fruit juice used in the study were purchased from different location in Bauchi. Some of them were brought from Wunti market, ATBU market, Yelwa Makaranta market, Yelwa Tudu market and Yelwa Kagadama. The sample consisting of two (2) orange juice, one (1) apple juice, one (1) strawberry juice, one (1) peach juice, one (1) apple and mango juice, and pineapple juice sample were tested within an hour after procurement.

B. *Total colony counts and isolation of microorganisms from Juice Sample*

Pour plate method was used to determine the total colony count using nutrient agar (Oxoid, UK). Serial dilution of samples was made ranging from 10^{-1} to 10^{-5} with sterile distilled water. 0.1ml of each dilution was poured on the nutrient agar medium and incubated at 37^0c for 24 hours.

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After incubation plates were observed for the presence of discrete colonies and the numbers of bacteria were estimated as colony forming unit per ml (CFU/ml). Bacteria isolates were identified using gram staining and biochemical tests.

C. Antimicrobial Susceptibility Testing

This was done using Kirby-Bauer disc diffusion method. Isolates were tested against seven commonly used antibacterial drugs on Mueller-Hinton agar with antibiotic discs (Oxoid). A single colony of each isolate was introduced into 2ml of Mueller-Hinton broth, incubated for 4 hours, and the culture turbidity was then adjusted to 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface. Antibiotic impregnated disc, (Gentamicin 10µg, Ceftazidime 30 µg, ciprofloxacin 5µg, amoxicillin 25µg, tetracycline 30µg, linezolid 30µg and erythromycin 15µg) were then placed onto the surface of the inoculated plates. After incubation, diameter of the zone of inhibition were measured in millimeters (mm) and interpreted as susceptible, intermediate and resistance.

S/N	ANTIBIOTIC	ABB.	DISC CONCENTRATION µg	R	I	S
1	Amoxicillin	AMO	25 **µg**	≤11	12 – 16	≥ 16
2	Ciprofloxacin	CIP	5 **µg**	≤15	16 – 20	≥21
3	Ceftazidime	CAZ	30 **µg**	≤14	15 – 17	≥18
4	Erythromycin	E	15 **µg**	≤13	14 – 22	≥23
5	Gentamicin	CN	10 **µg**	≤12	13 – 14	≥16
6	Linezolid	AZ	30 **µg**	≤12	13 – 17	≥18
7	Tetracycline	TE	30 **µg**	≤14	15 – 18	≤19

Table 1 : Details of Antibiotic Discs used during the study

Key: I = Intermediate, R= Resistance, S = Sensitive

III. RESULTS

Parameter	Total Bacteria cell count	Coliform	Faecal Coliform	Staphylococci
Maximum bacteria load anticipated	5.0×10^3	10	0	100
Maximum bacteria load permitted	1.0×10^4	100	0	1.0×10^3

Table 2: The Recommended Microbiological Standard for Juice, all number are as per ml of consumed (Gulf Standard, 2000)

Sample No	Type of juice	Sample area	Total variable count (TVC) CFU/ml
P – 1	Mango	Yelwa Makaranta	3.8×10^4
P – 2	Peach	Wunti	1.1×10^5
P – 3	Orange 1	ATBU Market	2.5×10^4
P – 4	Strawberry	Yelwa Tudu	2.9×10^4
P – 5	Apple	Yelwa Kagadama	2.0×10^4
P – 6	Apple/mango	Yelwa Makaranta	5.0×10^4
P – 7	Pineapple	Wunti	2.4×10^4
P – 8	Orange 2	ATBU Market	3.0×10^5

Table 3: Total Bacterial load in packaged juice sample

Bacterial Isolates	Colour on nutrient agar	Colour on mannitol salt agar	Colour on simmon agar	Gram reaction	Shape of isolate
P – MJ 1	Off-white	Yellowish	Off-white	Positive	Cocci in cluster
P – MJ 2	Off-white	Off-white	Mucoid transparent	Negative	Cocci
P – OJ 1	Bright yellow	Off-white	Bright yellow	Negative	Short rod
P – PJ 2	Orange	Yellowish	Off-white	Positive	Cocci in cluster
P – SJ	White (pale)	Mucoid transparent	Greenish	Negative	Long rods in chain
P – AMJ	Off-white	White	White	Negative	Cocci in cluster
P – PAJ	Mucoid transparent	Mucoid transparent	Off-white	Positive	Cocci in grape like branches
P – OJ 2	White (pale)	Mucoid transparent	Greenish colour	Negative	Long rods chain
P – AJ	Bright yellow	Yellowish	Brightly yellow	Positive	Cocci in tetrad
P – PJ 1	Off-white	Off-white	Greenish	Negative	Short rods.

Table 4: Cultural and microscopic characteristics of isolated colonies

KEYS: MJ- mango juice, OJ- orange juice, PJ- peach juice, SJ-strawberry juice, AMJ- apple & mango juice, PAJ- pineapple juice.

Isolate No	Isolate	Oxidase test	Catalase test	Coagulase test	Urase test	Indole test	Simmons citrate	Centrimide agar	Bacteria isolated
	P – MJ 1	-	+	+	-	-	-	-	Staphylococcus aureus
	P – MJ 2	-	+	-	-	+	-	-	*Escherichia coli*
	P – OJ 1	-	-	-	+	-	+	-	*Klebsiella spp*
	P – OJ 2	-	+	+	-	-	-	-	*Staphylococcus aureus*
	P – PJ 2	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
	P – SJ	-	-	-	-	+	-	-	*Escherichia coli*
	P – AMJ	-	-	-	+	-	+	-	*Klebsiella spp*
	P – PAJ	+	-	-	-	-	-	+	*Pseudomonas aeruginosa*
	P – AJ	-	+	+	-	-	-	-	*Staphylococcus aureus*
	P – PJ 1	+	+	+	+	-	-	+	*Pseudomonas aeruginosa*

Table 5: Biochemical characteristics of bacterial isolate from different juice.

	Isolate	CIP (5µg)	CAZ (30µg)	CN (10µg)	E (15µg)	LZD (30µg)	AML (25µg)	TE (30µg)
MJ	*Staphylococcus aureus*	S	R	S	I	R	S	R
MJ	*Escherichia coli*	S	R	I	S	I	R	R
OJ1	*Klebsiella Spp*	S	R	I	S	R	S	R
OJ2	*Pseudomonas aeruginosa*	S	I	S	I	S	R	R
PJ2	*Staphylococcus aureus*	S	S	I	R	R	S	R
SJ	*Escherichia coli*	S	R	R	S	I	S	R
AMJ	*Pseudomonas aeruginosa*	R	R	S	S	I	S	S
PAJ	*Klebsiella spp*	I	S	S	R	I	I	R
AJ	*Staphylococcus aureus*	S	S	I	R	S	S	I
PJ	*Pseudomonas aeruginosa*	S	R	I	I	R	S	S

Table 6: Antimicrobial susceptibility of bacteria isolated in package fruit juice

IV. DISCUSSION

Fruit juices offer numerous benefits yet, people are developing worries on their safety and quality especially due to the high numbers of brands in Nigeria. In this study, high microbial load range from 1.1×10^5 - 3.8×10^4 from various fruit juice tested. the bacterial contamination could be due to low quality material, contaminating processing equipment and environmental packaging material and untrained workers. The bacterial count was found to be low for some of the packaged fruit juice (peach) compared to some other(mango) this could be due to the fact that acidic conditions makes it unfavourable for microorganism to grow. Maximum permissible range for total viable count is 1.0×10^4 cfu/ml (Gulf standard, 2000).

The microorganism isolated in this study include S. *aureus* (30%), *P. aeruginosa* (30%), *Klebsiella* spp (20%), and *E. coli* (20%) respectively. These isolates have been found to be causative agent of food poisoning and intoxication (Kawo and Abdulmumini, 2009). None of the sample contained *Salmonella* and *Shigella spp* even though studies have reported the presence of *Salmonella* in fruit juice (Rahman *et al.*, 2011)

The result of the antibiotic susceptibility test presented on table 6 showed bacteria species having an intermediate, resistance to sensitive pattern of reaction to most of the antibiotic tested. All the bacteria isolated were 80% sensitive to ciprofloxacin (5µg). 70% are sensitive to amoxicillin (25µg) while it shows 70% resistance to tetracycline (30µg), 60% to ceftazidime(30µg). This resistance pattern shown could render these pathogens causes of serious health hazards due to ineffective treatment of the sufferers when these antibiotics are prescribed. *Klebsiella* spp and *E. coli* showed resistance to tetracycline. Stock and Wiedemann (2001), stated that *Klebsiella* spp. were naturally sensitive or intermediate to several penicillins, all tested aminoglycosides, quinolones, tetracyclines, trimethoprim, cotrimoxazole, chloramphenicol and nitrofurantoin. (Srinu *et al.*, 2012) has also reported that *E. coli* was sensitive to ciprofloxacin. There was 100% sensitivity of *S. aureus* isolates to Ciprofloxacin and Amoxicillin. *Pseudomonas aeruginosa* isolates showed varying pattern of resistance to the antibiotic discs.

V. CONCLUSION

The current study showed the bacteriological status of some available packaged fruit juice to ensure food safety for a precise control over public health risk. The average counts for bacteria of the packaged juice sample examined were above the maximum allowable unit in food to be marketed for consumption (10^3cfu/ml). These counts are suggestive of bacterial contamination and could be due to the handling processes since they are in liquid form which could have

contributed to the development as well as multiplication of these contaminants.

VI. RECOMMENDATION

It is essential for the personnel handling these juice preparations and manufacturing to be properly trained on safe fruit handling techniques. Quality control and assurance of fruits juice for human consumption is recommended in order to avoid any future bacteria pathogen outbreak. The vendors and consumers should be educated on the implication of food borne pathogen in fruit juice.

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