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New Insights into *Escherichia Coli* Associated Contaminants Inplicated in Locally Fried Plantain (Dodoikire) Sold at Ile Ife and Environs, Osun State, Nigeria

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Abstract

We investigated bacterial load of thirteen samples of locally fried plantain delicacy (dodoIkire) sold randomly around Ile- Ife, Osun State, Nigeria and its environment. Plate count and culture methods were used for evaluation process.

Sixteen bacterial isolates were obtained from the sample's sources. Plate count revealed that the bacterial load of the samples ranged from 5.60x108CFU/ml to 4.44x109CFU/ml. The sample sold at OUI Roundabout had the highest bacterial load of 4.44x109CFU/ml while the sample bought at Lagere had the lowest bacterial load of 5.60x108CFU/ml.

According to the biochemical tests result, six genera of bacteria were identified; Klebsiella spp (25%), Pseudomonas aeruginosa (25%), Yersinia spp (18.75%), E. coli (12.5%), Veillonella spp (12.5%) and Serratia spp (6.25%). Identified bacteria isolates were subjected to certain classes of antibiotics used Ciprofloxacin (5µg), Ceftazidime (30µg), Nitrofurantoin (300µg), Augmentin (30µg), Ofloxacin (5µg), Cefixime (5µg), Gentamicin (10µg), Cefuroxime (30µg). All isolates retrieved were 90% susceptible to oofloxacin (but was susceptible to the remaining antibiotics). Except Veillonella spp. that shows intermediate resistant strains factors. All isolates were resistant to augmentin antibiotic used. E. coli showed resistance to augmentin (but was susceptible to the remaining antibiotics). This finding necessitates monitoring policy and proper implementing measures, rules and regulations to be imposed on street food vendors so as to maintain standard hygienic procedures in preparation, cooking and handling of foods.

Keywords: *Escherichia Coli*, Dodoikire, Food Handler's Contamination, Antibiotic Resistance.

Introduction

Food handlers have been reported to greatly contribute to the dissemination and distribution of pathogens due in most cases to their low level of education, poor personal and environmental hygiene [1, 2]. Moreover, some of the food vendors are carriers of most of the enteric bacterial pathogens and they consequently introduce the pathogens into the food they handle [3, 4]. This emphasizes the importance of placing a priority on assisting street vendors in understanding the importance and the requirements of food safety.

Escherichia coli are considered the most commensally living microorganism in the alimentary tract of nearly all domestic and wild animals as well as human. Enteropathogenic E. coli organisms usually lead to severe diarrhea in infants and it may also be the causal organisms in appendicular abscess, peritonitis and cholecystitis [4, 6]. The Enterobacteriaceae is the most challenging bacterial contaminant to food products worldwide. Food-borne illnesses impact the entire world. In the United States, based on recent information from the Centers for Disease Control and Prevention, annual incidences of food-related diseases involve 76 million cases, of which only 14 million can be attributed to known pathogens. Recent estimates indicate that noroviruses, Campylobacter jejuni, and Salmonellaare the major causes of food-borne diseases. In addition, *Escherichia coli* O157:H7 and Listeria are important food related pathogens.

Food vendor services is on the increase and responsibility for good manufacturing practices of food such as good sanitary measures and proper food handling have been transferred to food vendors who rarely enforce such practices [7]. It has been reported that most food vendors in developing nations grossly lack formal knowledge of food preparation and hygiene [8-11]. Ready to eat foods are the status of foods being ready for imme-

diate consumption at the point of sale. Ready to eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment [12]. They can be described with different terms such as snacks, convenient, ready, instant and fast foods. In a study carried out in Cameroon, of 200 samples of ready to eat meats collected, (30%) were contaminated with E. coli, (23%) were contaminated with B. cereus, (19%) with S. aureus, (15%) with Salmonella spp. and (5%) with yeast and moulds (Djoulde, James and Bakary, 2015).

In Nigeria today, much interest is being placed on the consumption of non-homemade foods like snacks, food from restaurants or street foods. Ready to eat foods usually contain a number of ingredients which may or may not be cooked [13]. Ready to eat foods because of the fact that they are taken without further treatment are expected to be balanced nutritionally, healthy, easy to eat, provide quick energy and be of great taste.

However, excessive consumption of many sugary snacks has been attributed to incidences of obesity, diabetics and celiac diseases, especially the foreign snacks [14]. DodoIkire; being an example of a locally made ready to eat food is a popular age long over-ripe plantain-based snack, an example of a local snack that can be consumed to alleviate the spite of hunger in people. It is a specially fried plantain snack, which is commonly produce in Ikire town and its environs, in Osun State, western part of Nigeria, hence the name "dodoIkire". It fulfills the saying "waste not, want not" as fairly overripe plantain fruits are used to prevent them from waste [15].

Nutritionally, consumption of plantain-based products like dodoIkire promotes weight loss in obese individuals and caters to the calorific need of many developing countries [16]. In the bid of sellers to make more income, the trend in frying this "DodoIkire" has gone far from the era of prevention of wastage as it is now the practice to mix (adulterate) banana with plantain for the snack and then either wrapped and displayed in low density nylon or open plastic or metallic containers, with little or no attention to loss of sensory attributes and microbiological quality.

In this study we sourced to investigate the contamination of local fried plantain with *Escherichia coli* and determined the susceptibility trends of the isolates recovered with various antibiotics used [17].

Materials and Methods

Samples Collection

Fourteen (14) different samples were collected from different locations in Ile-Ife, Osun State such as different locations in OAU Ife (Butteries in Fajuyi and Moremi halls of residence, New market, BCH lab), Ibadan road, Asherifa, OUI gate, Toll gate and Lagere between the months of September and October 2018. They were checked for absence of tear to avoid contamination. They were then collected into nylons and labeled accordingly and immediately transported to the laboratory where it was analyzed within 24 hrs.

Enrichment and Culturing

At first, the first batch of sample brought were dissolved in sterile distilled water, shaken and then spread on EMB agar and incubated for 24hrs, but very little or no growth was observed using this method until enrichment using peptone water later adopted.

Serial Dilution and Plating

Serial dilutions of the enriched samples were carried out to reduce the microbial load to enable plate counting. The enriched sample was labeled as the "stock". 1ml of the stock was then pipette into another test tube containing 9ml sterile distilled water.

This was labeled as the "10-1 dilution". This process was repeated and the following dilutions were obtained: 10-2, 10-3, 10-4, 10-5, 10-6, 10-7 and 10-8. 0.1ml of the 10-6 and 10-8 dilutions were then plated on Nutrient agar for plate counting using the spread plate method. The plates were then incubated for 24hours. Also, 0.1ml of stock was measured into a petri dish containing EMB agar and spread using a glass spreader.

It was then incubated for 24-48hours in the incubator. Biochemical tests include Citrate test, SIM test, Catalase test, Methyl red Voges-Proskauer test, Oxidase test and Urease test were done to characterize and identify E. coli.

Antibiotics Susceptibility Testing

This was done using the agar disc diffusion method. Test tubes each containing 5ml sterile distilled water were prepared. The test tubes were inoculated with a 18-24-hour old culture till the water became turbid. Sterile swab sticks were then used to streak the inoculated water on the surface of Mueller Hinton Agar (MHA).

Eight antibiotics discs were placed on the agar with the codes; OFL, AUG, NIT, CPR, CAZ, CRX, GEN, CXM which are Ofloxacin (5µg), Augmentin (30µg), Nitrofurantoin (300µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg) respectively. The plate incubated at 350C for 24 hours were observed for zones of inhibition. The zones were measured in mm and compared to CLSI standard.

Results and Discussion

A total of twenty-five (25) bacteria were isolated during the study. The bacterial load and colony characteristics of organisms present in the samples were observed macroscopically on Nutrient agar and recorded as shown in Table 3.1 and the bacterial load of the samples were represented on a bar chart in Fig 1.

The isolated bacteria were stored in kryovials and were labeled according to the sequence of isolation and preserved in the refrigerator. Gram staining was carried out on the isolates and the observation was recorded in Table 3. From the table, it was observed that the isolates gotten from samples from Ibadan road contained both gram negative cocci and gram-negative rods. At other locations, only gram-negative cocci were observed.

Biochemical tests were performed on sixteen (16) of the unknown isolates and the presumptive identity of the isolates was recorded in Table 4. From the biochemical tests, six (6) genera of bacteria were isolated which includes; Klebsiella spp, Yersinia spp, Veillonella spp, Pseudomonas spp, Serratia spp and *Escherichia coli* with percentage prevalence of 25%, 18.75%, 12.5%, 25%, 6.25% and 12.5% respectively shown in table 5. This is represented on a pie chart in fig 2.

Antimicrobial susceptibility testing was then carried out on the isolates and result recorded in Table 6. Agar disc method was used using eight antibiotics disc. The antibiotics were; Ci-

profloxacin (5µg), Ceftazidime (30µg), Nitrofurantoin (300µg), Augmentin (30µg), Ofloxacin (5µg), Cefixime (5µg), Gentamicin (10µg) and Cefuroxime (30µg).

The study identified six (6) bacterial genera from the analyzed samples of dodoIkire. These genera include; Klebsiella sp., Yersinia sp., Veillonella spp, Pseudomonas spp, Serratia spp, *Escherichia coli.* The isolation of these bacteria from dodo Ikire agrees with the work of Adam and Moses that foods we eat are rarely sterile, if ever.

From table 1, plate count revealed the bacterial loads of dodoIkire sold at different locations around Ife. The bacterial load of the samples ranged from 5.60x108CFU/ml to 4.44x109CFU/ml indicating a relatively high bacterial load. The sample sold at Roundabout had the highest bacterial load of 4.44x109CFU/ml while the sample with the smallest bacterial load of 5.60x108CFU/ml was obtained from Lagere. The bacterial load for the samples is less than satisfactory but can still be acceptable for consumption. The high bacterial load may be due to inadequate preparation, unsanitary handling, improper packaging and storage amongst others.

Gram staining revealed gram negative rods and gram-negative cocci. Fourteen (14) of the sixteen (16) bacterial isolates were observed to be rod shaped while two (2) were observed to be cocci shaped. From this result, it was observed that samples obtained from Ibadan road contained both gram negative cocci and gram-negative rods. Therefore, this sample had more species diversity than samples from other locations in relation to bacteria shape. The other samples had only bacteria that were gram negative rods.

Biochemical characterization and identification of the bacteria isolates was done using: SIM (Sulfide Indole Motility) test, catalase test, MRVP (Methyl Red Voges-Proskauer) test, oxidase test, citrate test and urease test. From the results obtained, identified genera were; Klebsiella spp (25%), Yersinia spp (18.75%), Veillonella spp (12.5%), Pseudomonas spp (25%), Serratia spp (6.25%), *Escherichia coli* (12.5%). Klebsiella spp and Pseudomonas spp were observed to be the most abundant genera of the identified isolates.

Serratiaspp was observed to be the least abundant (Table 3). The prevalence of a higher percentage of Klebsiella spp and presence of Pseudomonas spp. in dodo Ikire was documented by Olagoke et al. The higher percentage of these bacteria could be attributed to the fact that they are ubiquitous in nature and can be found in food that require considerable handling during preparation. It could also be due to the poor handling on the part of the food handlers (e.g use of unhygienic cooking utensils and substandard packaging system.

The strains of E. coli were isolated from samples sold at Lagere which had the lowest bacterial load. Presence of E. coli in food is indicative of possible contamination by faecal organisms. It also reflects unsatisfactory hygienic condition during processing or handling or transportation or all of the aforementioned.

Antibiotics susceptibility profiling of the identified bacteria revealed that Ofloxacin was most effective on the bacterial isolates. It was effective against all bacteria isolates except Veillonella spp which was resistant against all antibiotics used and. Augmentin was the least effective against any bacteria isolate as all the bacterial isolates were resistant to it.

The presence of pathogen in foods is an index of poor hygiene practices. Food borne agents that may cause illness in ready-to-eat foods is of a significant risk to consumer health and their absence is of paramount importance in food safety. Health hazards due to food contamination by pathogens can be minimized by proper handling of food and good personal hygiene before, during and even after food processing or preparation. Although low numbers of pathogens in ready-to-eat products probably represent a very low risk to immunocompetent people, they are significant for the immunocompromised and vulnerable groups.

Table 1: Bacterial load & colonial characteristics of isolates obtained from samples at different locations on Nutrient Agar plate

Location	Dilution Factor	Microbial Load (CFU/ml)	Shape	Size	Color	Elevation	Margin	Edges	Surface
Fajuyi buttery	10^{-6}	$9x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$1.5x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Ibadan road	10^{-6}	$8.8x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$7.6x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Moremi Buttery	10^{-6}	$7x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$7.2x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Bch lab	10^{-6}	$3.2x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$2.6x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Oau market	10^{-6}	$9x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$1.5x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Asherifa	10^{-6}	$6.1x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$1.9x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
Oui gate	10^{-6}	$9.5x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet
	10^{-8}	$7x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Wet

Table 2: Bacterial load & colonial characteristics of isolates obtained from samples at different locations on Nutrient Agar plate (continued)

Location	Dilution factor	Microbial load (cfu/ml)	Shape	Size	Color	Elevation	Margin	Edges	Surface
Round about	10^{-6}	$9.1x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$8.8x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
Toll gate i	10^{-6}	$9x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$8.5x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
Toll gate ii	10^{-6}	$9.2x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$7.6x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
Mayfair	10^{-6}	$2.3x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$1.6x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
Lagere	10^{-6}	$1.8x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$1.1x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
Sabo	10^{-6}	$2x10^{7}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth
	10^{-8}	$1.5x10^{9}$	Circular	Small	Cream	Raised	Entire	Smooth	Smooth

Table 3: Bacterial load of isolates obtained from samples at different locations on Nutrient Agar plate

Location	Mean microbial load (x109cfu/ml)
Fajuyi buttery	0.80
Ibadan road	3.84
Moremi buttery	3.64
BCH lab	1.32
OAU market	0.80
Asherifa	0.98
OUI gate	3.55
Round about	4.44
Toll gate I	4.30
Toll gate II	3.85
Mayfair	0.81
Lagere	0.56
Sabo	0.76

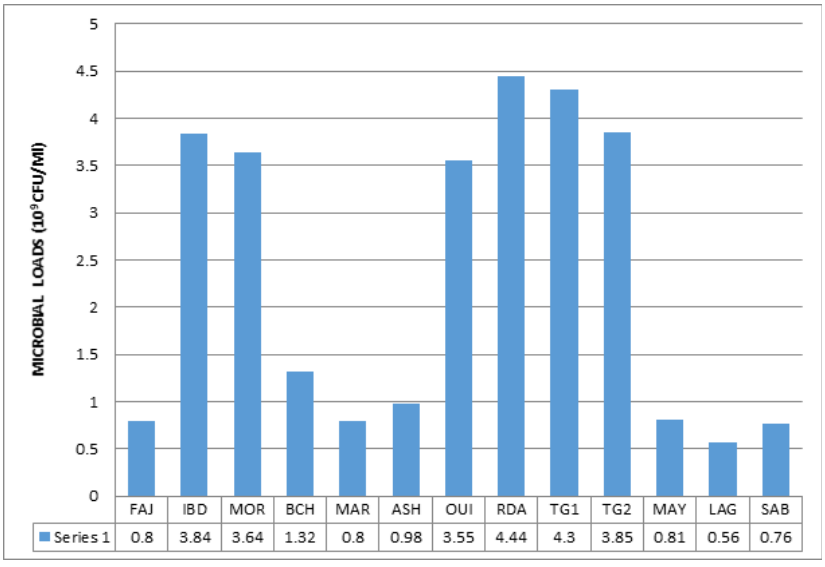

Figure 1: A bar chart showing bacterial load on Nutrient Agar plates of samples sold at different locations.

KEY: FAJ – Fajuyi buttery, IBD – Ibadan road, MOR – Moremi buttery, BCH – Biochemistry lab, MAR – OAU Market, ASH – Asherifa, OUI – OUI gate, RDA – Round about, TG1 – Toll gate1, TG2 – Toll gate2, MAY – Mayfair, LAG – Lagere, SAB – Sabo.

Table 4: Biochemical characteristics and identification of bacterial isolates

Isolates	Sulfide	Indole	Motility	Catalase	MR	VP	Oxidase	Citrate	Urease 24hours	Urease 24hours	Suspected organisms
E1A	_	_	_	+	+	_	+	+	+	+	Klebsiella spp.
E1B	_	_	_	+	+	_	+	+	+	+	Klebsiella spp.
E2A	_	_	_	+	+	_	_	+	_	_	Yersinia spp.
E2B	_	_	_	+	+	_	_	+	_	+	Yersinia spp.
E3A	_	_	_	_	_	+	+	_	_	_	Veillonella spp.
E3B	_	_	_	_	_	+	+	_	_	_	Veillonella spp.
E3C	_	_	_	+	_	_	+	+	_	+	Pseudomonas spp.
E3D	_	_	_	+	_	_	+	+	_	+	Pseudomonas spp.
E4C	_	_	+	_	_	+	_	_	+	+	Serratia spp.
E4D	_	_	_	+	_	+	+	_	+	+	Yersinia spp.
E5A	_	_	_	+	_	_	+	+	_	_	Pseudomonas spp
E5D	_	_	+	+	_	_	+	+	_	_	Pseudomonas spp.

Table 5: Biochemical characteristics and identification of bacterial isolates(continued)

Isolates	Sulfide	Indole	Motility	Catalase	MR	VP	Oxidase	Citrate	Urease 24hours	Urease 4days	Suspected organisms
E6A	_	+	_	+	_	+	_	+	_	_	Klebsiella spp.
E6B	¬_	+	_	+	+	_	_	_	_	_	*Escherichia coli*
E7B	_	+	_	+	+	_	_	_	_	_	*Escherichia coli*
E7C	_	+	_	+	_	+	_	+	_	_	Klebsiella spp.

KEY: + = positive, - = negative

Table 6: Percentage prevalence of identified bacterial isolates obtained from the samples of dodoIkire

Isolates	Percentage Prevalence (%)
Klebsiella spp.	25
Yersinia spp	18.75
Veillonella spp	12.5
Pseudomonas spp	25
Serratia spp	6.25
E. coli	12.5
Total percentage	100

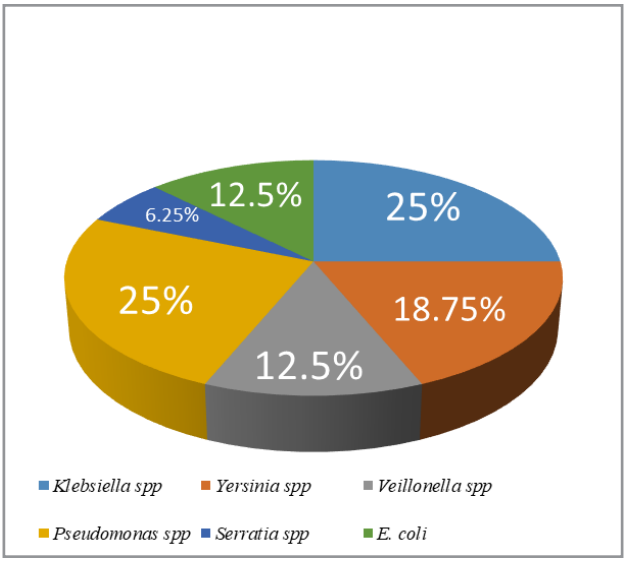

Figure 2: A pie chart showing the percentage prevalence of bacterial isolates obtained from samples of dodoIkire

Table 7: Antibiotics susceptibility pattern of the isolates

Antibiotics discs Isolates		Antibiotics	with	Zones	of	Inhibition	in	MM
	OFL	AUG	NIT	CPR	CAZ	CRX	GEN	CXM
Klebsiella spp.	21(S)	0 (R)	25 (S)	22 (I)	0 (R)	0 (R)	24 (S)	0 (R)
Klebsiella spp.	22 (S)	0 (R)	23 (S)	25 (I)	0 (R)	0 (R)	22 (S)	0 (R)
Yersinia spp.	24 (S)	0 (R)	25 (S)	28 (I)	0 (R)	0 (R)	26 (S)	0 (R)
Yersinia spp.	29 (S)	0 (R)	21 (S)	31 (S)	0 (R)	0 (R)	24 (S)	0 (R)
Veillonella spp.	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Veillonella spp.	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
Pseudomonas spp.	29 (S)	0 (R)	24 (S)	34 (S)	0 (R)	0 (R)	25 (S)	0 (R)
Peudomonas spp	30 (S)	0 (R)	23 (S)	33 (S)	0 (R)	0 (R)	26 (S)	0 (R)
Serratia spp.	29 (S)	0 (R)	27 (S)	35 (S)	0 (R)	27 (S)	28 (S)	17 (I)
Yersinia spp.	32 (S)	0 (R)	28 (S)	30 (I)	28 (S)	30 (S)	30 (S)	19 (S)
Peudomonas spp.	33 (S)	0 (R)	26 (S)	34 (S)	27 (S)	0 (R)	26 (S)	21 (S)

Table 8: Antibiotics susceptibility pattern of the isolates (continued)

Antibiotics discs Isolates		**Antibiotics**	**With**	**Zones**	**Of**	**Inhibition**	**In**	**MM**
	OFL	**AUG**	**NIT**	**CPR**	**CAZ**	**CRX**	**GEN**	**CXM**
Pseudomonas spp.		Z 29 (S)	27 (S)	31 (S)	22 (S)	0 (R)	24 (S)	0 (R)
Klebsiella spp.	27 (S)	0 (R)	24 (S)	31 (S)	21 (S)	0 (R)	22 (S)	19 (S)
E. coli	31 (S)	0 (R)	25 (S)	30 (I)	29 (S)	20 (S)	24 (S)	24 (S)
E. coli	31 (S)	0 (R)	26 (S)	31 (S)	21 (S)	21 (S)	22 (S)	30 (S)
Klebsiella spp	28 (S)	0 (R)	27 (S)	36 (S)	26 (S)	18 (I)	22 (S)	30 (S)

KEY: CPR – Ciprofloxacin (5ug), CAZ – Ceftazidime (30ug), NIT – Nitrofurantoin (300ug), AUG – Augmentin (30ug), OFL – Ofloxacin (5ug), CXM – Cefixime (5ug) GEN – Gentamicin (10ug) CRX – Cefuroxime (30ug)
S –Susceptible
I – Intermediat, R – Resistant

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