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PREVALENCE AND RESISTANCE PROFILE OF STRAINS OF BACTERIA ISOLATED FROM MEALS IN N'DJAMENA, CHAD

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ABSTRACT
Poorly preserved food is a favorable environment for the growth of microorganisms. This study is conducted to evaluate the microbiological and hygienic characteristics of household meals in the city of N'Djamena, Chad. Total of 180 samples were taken for the study. The samples consisted of sorghum, maize, rice, meat, fish, milk, okra and bean leaves. Standardized methods have been used to perform microbiological analyzes. The manual and automated methods with the Vitek Compact 15 were used to identify and characterize the seeds. For antibiotic susceptibility testing, antibiotics were chosen based on their use. The results showed that there is a significant difference ($p = 0.02$) in the contamination between fresh meat and dried okra sauce with fresh meat by *Escherichia. coli* (40%) and *Staphylococcus aureus* (13.3%). There is also a significant difference ($p=0.001$) of pathogen contamination between roasted beef and "Rhaïb" milk by *Streptococcus agalactiae* (33.3%) and *Staphylococcus aureus* (26.7%). No Salmonella was detected on all the foods collected. Compared to the susceptibility test of isolated organisms, multiresistant bacterial strains were observed. Good practices of hygiene and conservation of food are needed to ensure food quality.

KEYWORDS: Bacteria, Prevalence, Resistance, Meal, Food.

INTRODUCTION
The meal is all food and beverages to be consumed at one time in a more or less fixed time, but in some households particularly in Africa, taking into account dietary habits and socio-cultural factors, the same meal can be kept and consumed several times. This practice exposes foods from different meal to contamination by pathogenic bacteria because leftover meals are not reheated or reheated to low temperatures for consumption (Bessimbaye et al., 2013). The origin of food contaminations by pathogenic bacteria varies according to the nature of the product and its mode of production and processing. The contamination of the meal and the main foods consumed by pathogenic microorganisms in households may be of endogenous or exogenous origin (Bsadjo et al., 2015). Also, the unhealthy nature of these foods comes from non-compliance with good hygiene practices in their processing, cooking, preservation or sale (Mufizur et al., 2014; Ire, 2016 ; Djibrine et al., 2018). Poor practices including an unacceptable level of food hygiene is a risk indicator for foodborne illness (Lee et al., 2012 ; Djekic et al., 2014). The World Health Organization (WHO) indicates that foodborne diseases reduce economic growth in developed countries and developing countries and significantly degrade health (WHO, 2013). However, to prevent contamination, it would be sufficient to apply good hygiene in the handling, preparation, service and storage of food (Djibrine et al., 2017). In Chad, meals are dominated by a large proportion of cereals (millet, sorghum, maize, rice). They are prepared as a paste or cereal patties, accompanied meat sauce, fish and vegetables. But these foods are prepared and often kept in very poor hygienic conditions (Djibrine et al., 2018). As a result, consumers may develop foodborne illness in relation to microbial contamination (Tidjani et al., 2016). Unfortunately, once infected, patients often resort to self-medication through the misuse of antibiotics, which leads to a resistance of the germs to these drugs, and the emergence of the

antimicrobial resistance phenomena which results in a therapeutic failure. The purpose of this study is to isolate pathogenic microorganisms in meals usually consumed in households, and carry out the antibiotic susceptibility test commonly used in agriculture, breeding, and health in Chad. This work is a tool to raise awareness of good hygiene practices in the handling and preservation of meals and will also help the effective and safe management of antibiotics.

MATERIAL AND METHODS

Areas, period and type of study

This is a prospective and analytic study conducted in districts (1st, 6th and 7th Arrondissements) of the city of N'Djamena, capital of Chad. These are among the most populated neighborhoods of N'Djamena (INSEED, 2009): Karkandjé, Milezi (1st Arrondissement); Moursal, Chagoua (6th Arrondissement); Dembé, Atône, Boutalbagara (7th Arrondissement). The choice of these neighborhoods is part of the desire to have a better representation of the sampling. The research was conducted from January 2016 to July 2018 at Laboratory of Food and Nutrition Sciences (LARSAN) of the Faculty of Human Health Sciences, University of N'Djamena.

Characteristics of household foods and sampling

The food sampling was carried out on the basis of individual interviews in French or local languages, if necessary using a questionnaire in one household out of four at random. All foods were listed and the most common foods were chosen for testing. The sample size was estimated by taking into account randomly selected households (n_1 = 12) and the number of samples per household, ie 5 samples for each meal period (3 meals per day) (n_2 = 3 x 5 = 15). A total of 180 samples (n_1 x n_2 = 12x15 = 180) were made. The types of food concerned and often consumed in households are cereals (sorghum, maize, and rice), animal products and their derivatives (meat, fish, and milk) and vegetables (okra and bean leaves).

Household meals are collected at 2.30 pm when the meals of the day are prepared hot and ready to eat at 8 pm and 7 am for the rest of the same meal kept for eating. In addition to hot meals, samples of fresh milk, fresh meat, fresh fish and fresh vegetables were collected. For the same household, 15 samples were taken at the rate of 5 samples according to the different meal times. The samples of 500 g (for solid products) and 500 ml (for liquid products) were collected in sterile sachets or vials, sent to the Research Laboratory of Food Science and Nutrition (LARSAN) at 4 °C and analyzed within a few hours. Samples of fresh fish and fresh meat were made when the housewife cut them for preparation. Especially for catfish, branches were taken for bacteriological analyzes.

Bacteriologiacal analysis

Microbiological analyzes identified the following germs: *Escherichia coli*, *Streptococcus agalactiae*, *Vibrio fluvialis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The bacteriological analysis were carried out respecting the standard conditions of food microbiology.

Isolation and identification of the pathogens sought

Two methods were used: the manual method and the automated method with the Vitek Compact 15.

Manual method: Microorganisms were searched for using the standardized standard reference methods reported in the work of Clarence et al. (2009). Other supports (AFNOR, 2002) were used. The revivification of microorganisms was made by peptone water at 45 minutes at room temperature. The preparation of the decimal dilutions was made according to standard NF V 08-010. Decimal dilutions were obtained by adding one ml of each of the previous ones, using a new graduated pipette, to the 9 ml contained in the tubes. The following agars were used for isolation of germs: Hektoen, Mac Conkey, Chapman. After 18 to 24 hours of incubation bacteriological incubator at 37°C, The yellow colonies on the agar Hektoen and colonies with metallic reflections were suspicious of *Escherichia coli.* On Chapman agar the colonies of *Staphylococcus aureus* presented yellow color. TCBS medium was used for isolation of *Vibrio fluviatus*. Petri dishes containing seeded TCBS medium were incubated at 37 ° C in an oven for 18-24 hours. Yellow colonies (sucrose +) on this medium were suspected for identification of *Vibrio fluviatus.* To perform the antibiogram, we used the Muller Hinton medium (MH) by considering the Kirby Bauer technique (Bauer et al., 1966).

The automated method (Vitek Compact 15) was used to confirm the manual method: The system includes the Vitek® 2 Compact instrument, a computer (workstation) and a printer. The software supplied with the Vitek® 2 Compact system includes data analysis and data management programs. As part of this study, we used identification cards as well as AST antibiogram cards. AST cards were used to determine the antibiotic susceptibility of the germs. The antibiogram of Vitek® 2 uses the determination of the minimum inhibitory concentration (MIC). Each Vitek® 2 AST card contains 64 microwells. A control wells containing only the culture medium is present on all cards; the other wells contain pre-established concentrations of a given antibiotic and a culture medium. The Vitek® 2 instrument controls growth in each well on the map for a set period of time (up to 18 hours for bacteria). At the end of the incubation cycle, the MIC values are determined for each antibiotic present on the map. GN (Gram negative) and GP (Gram positive) cards each have more than 40 biochemical tests. The final results are obtained in about 10 hours for the bacteria.

Choice of antibiotic discs to test

Antibiotics were chosen based on their current and abusive use in human, animal and agricultural health. Several antibiotics (BioRad, Marnes-la-coquette, France) were tested. These are: Aminopenicillin: amoxicillin (AMX: 25 µg), Amoxicillin / clavulanic acid (AMC: 20-10µg) Ampicillin (10 µg), Oxacillin 5µg, Penicillin G (benzylpenicillin); Cephalosporins: cephalothin (30 µg), ceftriaxone (CRO: 30 µg), Carbapenems: imipenem (IPM: 10 µg); Gentamicin (GEN: 15 µg); Tetracycline (TET: 30 µg) doxycycline (DO 30 µg); Chloramphenicol (CHL: 30 µg); Nalidixic acid (NAL: 30 µg) Ciprofloxacin (CIP: 5 µg), Rifampicin (RAM: 30µg); Vancomycin (VNC 30µg) and Fusidic acid (FAD 10 µg) (Fucidine).

Data processing

The collected data were captured and analyzed using the Excel 2013 software. They were processed taking into account the bacterial aetiology, the type of food, the living conditions of the households and the mechanisms of resistance of the bacteria to antibiotics. The Chi-square test (χ^2) was used to compare the qualitative variables with a significance level of 5%.

RESULTS

Survey of Household

With regard to household characteristics, only 25% of households surveyed have electricity with untimely cuts that do not allow the proper conservation of meals.

Prevalence of pathogens isolated in household meals

Table 1 shows the prevalence of pathogens isolated in household meals. Contamination was observed in almost every meal. Most leftover meals eaten around 19:00 are not warmed up to be eaten. This practice crowned with ignorance causes the contamination of meals by roaches, rats and other insects. A significant difference ($p = 0.02$) in contamination was observed between fresh meat and dried okra sauce with fresh meat by *Escherichia coli* (40%) and *Staphylococcus aureus* (13.3%). In shorgo patties and corn samples, *Escherichia coli* was found with respective rates of 6.7% and 13.13%. There is also a significant difference ($p = 0.001$) in pathogen contamination between beef and Rhaïb (milk) by *Streptococcus agalactiae* (33.3%) and *Staphylococcus aureus* (26.7%). *Staphylococcus aureus* is also found in 26.7% of samples of dried okra sauce. Only fresh fish (catfish) contains *Vibrio fluvialis*. Also the results indicate that 3.33% of fresh meat samples are contaminated with *Pseudomonas aeruginosa*. On the other hand all the searched germs were not found in the rice samples. Similarly, no salmonella was detected in all the samples of the foods collected.

Sensitivity test of bacterial agents isolated from meals in households with antibiotics

Table 2 presents the results of the susceptibility test of isolated microorganisms. *Escherichia. coli* showed a resistance of about 60% to betalactamines (MIC>250) and a sensitivity of about 85% (MIC = 0.4) to Quinolones and Fluoroquinolones. *Pseudomonas aeruginosa* developed resistance in several families but was found to be 100% sensitive to fourth generation Quinolones, Fluoroquinolones and cephalosporins (CAZ). *Streptococcus agalactiae* and *Staphylococcus aureus* had developed a resistance of about 71% (MIC>200) to oxacillin. In contrast, *Staphylococcus aureus* was much more resistant to vancomycin (MIC>252) (glycopeptides) whereas *Streptococcus agalactiae* were resistant to 28.6% (significant difference: $x^2 = 74.399$, dd1 = 1, $p = 0.001$). *Vibrio fluvialis* was 100% sensitive to doxycycline and tetracycline (Cycline) (MIC = 0.5), amoxicillin (MIC = 0.33), and amoxicillin plus clavulanic acid (MIC = 0.37). On the other hand, it was sensitive to ciprofloxacin (fluoroquinolones) but resistant to nalidixic acid (quinolones). If we consider the minimum inhibitory concentration (MIC), almost all the germs sought have more or less developed resistance to some commonly used antibiotics.

Table 1: Prevalence of Isolated Pathogens in Household Meals.

Meal	Number of sprouts per meal (%)				
	E. coli	*S. agalactiae*	*V. fluvialis*	*S. aureus*	*P.aeruginosa*
Corn ball (n = 15)	1 (6.7%)	0 (0)	0 (0)	0 (0)	0 (0)
Sorghum ball (n = 15)	2 (13.3%)	0 (0)	0 (0)	0 (0)	0 (0)
Rice Ball (n = 15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fresh beef (n = 15)	6 (40%)	0 (0)	0 (0)	2 (13.3%)	2 (13.3%)
Dried okra with fresh beef (n = 15)	5 (33.3%)	0 (0)	0 (0)	1 (6.7%)	0 (0)
Dried okra with dried fish (n = 15)	3 (20%)	0 (0)	0 (0)	4 (26.7%)	0 (0)
Fresh fish (Catfish) (n = 15)	0 (0)	0 (0)	1 (6.7%)	0 (0)	0 (0)
Bean leaf with sesame flour (n = 15)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Kissar (n = 15)	3 (20%)	0 (0)	0 (0)	1 (6.7%)	0 (0)
Beef grilled meat (n = 15)	3 (20%)	0 (0)	0 (0)	2 (13.3%)	0 (0)
Rhaïb milk (n = 15)	3 (20%)	5 (33.3%)	0 (0)	4 (26.7%)	0 (0)
(n = 15)	5 (33.3%)	2 (13.3%)	0 (0)	3 (20%)	0 (0)
Total (n = 180)	**31(17.1%)**	**7 (4%)**	**1 (0.5%)**	**17 (9.4%)**	**2 (1.1%)**

E. coli = Escherichia coli, S. agalactiae = Streptococcus agalactiae, S. aureus = Staphylococcus aureus, P.aeruginosa= Pseudomonas aeruginosa

Table 2: Sensitivity test of bacterial agents isolated from antibiotic meals.

ATB	Bacterial agents									
	E. coli (n = 31)		*V. fluvialis* (n = 1)		*S. agalactiae* (n = 7)		*S. aureus* (n = 17)		*P. aeruginosa* (n = 2)	
	n (% (R +I))	n (% S)	n (% (R +I))	n (% S)	n (% (R +I))	n (% S)	n (% (R +I))	n (% S)	n (% (R +I))	n (% S)
PEN	-	-	-	-	3 (43)	4 (57.1)	15 (88.2)	2 (11.8)	-	-
AMP	27 (87.1)	4 (13)	-	-	2 (28.6)	5 (71.4)	13 (76.5)	4 (23.5)	2 (100)	0 (0)
AMX	24 (77.4)	7 (22.6)	0 (0)	1 (100)	3 (43)	4 (57.1)	-	-	2 (100)	0 (0)
AMC	14 (41.2)	17 (55)	0 (0)	1 (100)	-	-	-	-	2 (100)	0 (0)
OXA	-	-	-	-	5 (71.4)	2 (28.6)	12 (70.6)	5 (29.4)	-	-
CAZ	11 (35.5)	20 (67.5)	-	-	1 (14.3)	6 (86)	10 (59)	7 (41.2)	0 (0)	2 (100)
CRO	15 (48.4)	16 (51.6)	-	-	2 (28.6)	5 (71.4)	8 (47)	9 (53)	1 (50)	1 (50)
IPM	3 (9.7)	28 (90.3)	-	-	0 (0)	7 (100)	3 (18)	14 (82)	0 (0)	2 (100)
GMN	17 (55)	14 (45)	-	-	3 (43)	4 (57.1)	7 (41.2)	10 (59)	0 (0)	2 (100)
TOB	5 (16.1)	26 (84)	-	-	0 (0)	7 (100)	3 (18)	14 (82)	0 (0)	2 (100)
TET	19 (61.3)	12 (38.7)	0 (0)	1 (100)	4 (57.1)	3 (43)	11 (64.7)	6 (3.5)	2 (100)	0 (0)
DO	-	-	0 (0)	1 (100)	4 (57.1)	3 (43)	7 (41.2)	10 (59)	-	-
CHL	26 (84)	5 (16.1)	0 (0)	1 (100)	4 (57.1)	3 (43)	14 (45.2)	4 (23.5)	2 (100)	0 (0)

VCN	-	-	-	-	2 (28.6)	5 (71.4))	12 (70.6)	5 (29.4)	-	-
FAD	-	-	-	-	-	-	0 (0)	31 (100)	-	-
NAL	8 (26)	23 (74.2)	1 (100)	0 (0)	1 (14.3)	6 (86)	3 (18)	14 (82)	0 (0)	2 (100)
CIP	2 (6.4)	29 (93.5)	1 (100)	0 (0)	7 (100)	0 (0)	2 (11.8)	15 (88.2)	0 (0)	2 (100)
RAM	-	-	-	-	2 (28.6)	5 (71.4)	7 (41.2)	10 (59)	-	-

S. agalactiae = *Streptococcus agalactiae*, *S. aureus* = Staphylococcus aureus, ATB = antibiotic, %(R+I)= resistance + intermediate rate, (% S) = sensitivity rate, AMP = ampicilline, AMX = Amoxicilline, AMC = amoxicillin + clavulanic acid, CAZ = Ceftazidime, CRO = ceftriaxone, IMP = imipénème, OXA oxacillin, CIP = ciprofloxacin, FAD = fusidic acid, TET=Tetracyclin, DO = Doxycyclin, GMN = gentamycin, NAL = Nalidixic acid, CHL = chloramphenicol, VCN = vancomycin, PEN = penicillin, RAM = rifampicin, (-) = ATB non required for testing.

DISCUSSION

Microbiological analysis

These results show that foods prepared, poorly preserved and handled under poor hygienic conditions can harbor pathogenic germs. Regarding the conservation surveys, the study shows that in 75% of households poorly maintained meals without any protection against pests; these foods are reheated and eaten in the morning. Preservation requirements such as refrigerators, freezers and power cuts are often not ensured. These observations were made by Barro et al. (2007).

Microbiological analysis revealed the presence of *Escherichia coli* in all types of meals except samples of rice, fresh fish and bean leaves. Other studies (Christison et al., 2007; Ilboudo et al., 2009; Ahoyo et al., 2010) have also found this germ in meat, sandwiches or pasta. This may well be evidence of poor hygiene practices of food handlers who rarely wash their hands with soap before eating or after leaving the toilet. *Escherichia coli* is a commensal host of intestinal flora. It is a bacillus mobility peritrichous (Darnton et al., 2007), this germ is an indicator of faecal contamination as highlighted in several studies (Barro et al, 2007, Djibrine et al, 2018). *Staphylococcus aureus* was isolated from fresh meat samples, fresh fish, dried okra with dried fish, grilled meat and different types of milk. Another study (Le Loir et al., 2003) points out that foods usually contaminated with *Staphylococcus aureus* are milk, cream, pastries, butter, ham, cheese, sausages, canned meats, salads, cooked meals and sandwich fillings. Several other studies conducted in Africa and around the world have shown that meat products, cereal products, dairy products, local beverages, vegetables, etc. are contaminated with pathogens (Okareh et al., 2015 ; Bagré et al., 2017). Pathogenic microorganisms involved in these foods include *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, coliforms, yeasts and molds at different levels (Kasse et al., 2014 ; Bagré et al., 2014 ; Bsadjo et al., 2014 ; Okareh et al., 2015). The authors Ranjbar et al. (2018), Ahoyo et al. (2010) also detected various serotypes of *Escherichia coli*. Compared with *Staphylococcus aureus*, Lidiane Soares Pereira et al. (2017), indicates that this germ is commonly involved in food poisoning due to the production of toxins responsible for animal and human disease. The results of the study also show us the germs *Streptococcus agalactiae* with a high proportion in milk (33.3%) than in other types of food. Another germ, *Streptococcus agalactiae*, was found in Rhaïb milk with a considerable proportion (33.3%). *Streptococcus agalactiae* is an optional facultative anaerobic gram-positive bacterium. Humans and cattle are the main reservoir hosts of this bacterium (Barkema et al., 2009). This pathogen is sometimes isolated from dairy products or other agri-food products. Here again we can say that deficiencies in personal hygiene could justify the presence of these germs in the milk samples. Regarding germ *Pseudomonas aeruginosa*, it was detected only in fresh meat samples. Other studies (Bailly et al., 2012) confirm that this germ is mainly identified as an indicator of alteration of fresh meat and milk. It can be identified immediately after slaughter on meat carcasses (Salifou et al., 2013). The results of this study also show that a sample of fresh fish is contaminated with *Vibrio fluvialis*. The presence of Vibrio in the fish sample is thought to be due to the aquatic ecology which constitutes a favorable environment for the development of this virus. Furthermore (Traoré et al., 2014) isolated *Vibrio cholerea* in fish samples. Salmonellae were not found in all samples. Our results are similar to those of Millogo et al. (2018).

Other studies (Baba-Moussa et al., 2006, El Marnissi et al., 2012) conducted in food did not report the presence of this germ. On the other hand other authors have found Salmonella (Nywira Wahome and al., 2014, Abba et al., 2017). Salmonellae were also detected in uncooked dried fish samples in Chad (Gamané et al., 2018).

Antibiogram

The resistance of pathogens to antibiotics is a permanent and urgent concern in both developed and developing countries. Recent decades have seen the emergence of multidrug-resistant bacterial strains against which antibiotics have become totally ineffective (Bonny et al., 2014). These treatment failures have serious health consequences, damage the economy and also compromise the health care gains of society. In this study, Aminoglycosides (GMN, TOB) showed an average 35.5% resistance to *Escherichia coli* strains. Our results (26%) corroborate those of Bodering et al. (2017) who found resistance with a 23.08% level of *E. coli.* in samples from poultry farms in the cities of N'Djamena and Doba in Chad. However, Toudji et al. (2017) emphasized in their work a resistance of *Escherichia coli* to beta-lactams and other antibiotics, which would undoubtedly complicate the authors' treatment of patients. *Pseudomonas aeruginosa* germs have also developed resistance in several families of antibiotics. This critical situation of the pyocyanic and multiple resistance of *Pseudomonas aeruginosa* strains could be explained by the misuse of antibiotics (Bessimbaye et al., 2013; Nyawira Wahome et al., 2014). *Streptococcus agalactiae* and *Staphylococcus aureus* had developed resistance to oxacillin, with an even more significant resistance to *Staphylococcus aureus* vis-à-vis vancomycin. In Benin, a study on the bacteriological quality of foods sold in fast food restaurants on the university campus and to determine the frequency of multi-resistant bacteria (BMR) isolated. The following bacteria have been identified: *Escherichia coli*, Salmonella, *Staphylococcus aureus* and Enterococcus. Serotype *Escherichia coli* O157 and *Escherichia coli* strains of broad-spectrum beta lactamase (ECBLSE) were investigated; 74% of the samples are contaminated; 325 strains of the desired bacteria were isolated, of which 116 (36%) were BMRs including 44% of ECBLSE, 32% of methicillin-resistant *Staphylococcus aureus* and 24% of vancomycin-resistant Enterococci

(Ahoyo et al., 2010). Cautious use of drugs and improved food hygiene practices were the subject of recommendations to protect the public from the risk of acquiring infections caused by *Staphylococcus aureus* (Haftay et al., 2018). Also, several studies (Islam, 2009; Quilici et al., 2010; Ndoutamia et al., 2014) have shown that the anarchic use of antibiotics causes bacterial resistance to these drugs. Laurencin (2012) emphasizes that these resistances are the phenotypic expression of genetic modifications. Under these conditions and in case of infection, these antimicrobial resistance phenomena could be at the origin of multiple resistances with consequent therapeutic failure.

CONCLUSION

This study allowed us to determine the prevalence of pathogenic bacteria responsible for gastrointestinal disorders, to link the level of contamination of meals with the unhealthy environment and to carry out the sensitivity test of antibiotics commonly used in Chad. The impact of the abuse of antibiotics on the susceptibility of pathogenic bacteria to meals consumed in the city of N'Djamena has been highlighted. Contamination was observed in almost every meal. Poor conservation practices crowned with ignorance cause contamination of meals by pests. A significant difference in contamination was observed between fresh meat and dried okra sauce with fresh meat by *Escherichia coli* 6 (40%) and *Staphylococcus aureus* 2 (13.3%). There is also a difference in the degree of pathogen contamination between roasted beef and Rhaïb milk by *Streptococcus agalactiae* 5 (33.3%) and *Staphylococcus aureus* 4 (26.7%). No salmonella was detected on in all type of foods collected. The resistance of pathogens to antibiotics has also been demonstrated. Antibiotic resistance is a permanent and urgent concerned in both developed and developing countries.

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