African Journal of Microbiology Research

Volume 11 Number 46 14 December, 2017

ISSN 1996-0808

Academic Journals

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African Journal of Microbiology Research

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academicJournals

Vol. 11(46), pp. 1649-1656, 14 December, 2017
DOI: 10.5897/AJMR2017.8746
Article Number: 3F4D74C67083
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Sanitization protocols applied to commercial restaurants: Effects on natural contaminant microbiota and *Salmonella enterica* Enteritidis adhered on tomatoes

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Received 20 October, 2017; Accepted 13 November, 2017

Sanitization is considered as essential for the microbial control of vegetables. The aim of this study was to evaluate the sanitization procedures used in commercial restaurants located in Vitória, Brazil. The efficacy of these sanitization procedures in reducing the presence of natural microbiota and *Salmonella enterica* Enteritidis cells on tomatoes were evaluated. All the restaurants in this study applied the sanitization methods using containers for diluting the sanitization solution. After the sanitization treatments, a reduction in mesophilic aerobic counts, yeasts, moulds and *S. enterica* occurred in all the treatments. A higher reduction in microorganisms was observed after treatment with 2% acetic acid. There was no significant difference between tomatoes treated with a sodium dichloroisocyanurate solution and sodium hypochlorite for all microorganisms which were evaluated. Chlorinated compounds are the most used products but a limitation in microbial inactivation was observed in this study.

Key words: Disinfection, tomatoes, *Salmonella enterica* Enteritidis, acetic acid, quality control, sanitization protocols.

INTRODUCTION

Dietary consumption of vegetables and fruits has health benefits, including to avoid or decrease the possibility of developing several chronic diseases (Bang et al., 2017). The benefits related to fresh cut products have contributed to an important increase in the consumption of ready-to-eat vegetables (Callejón et al., 2015; Bang et

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al., 2017). However, the consumption of raw or minimally processed fruits and vegetables can be a route for the transmission of foodborne illnesses (Park et al., 2013; Prado-Silva et al., 2015). Among the multiple failures in food services, the development of foodborne illness outbreaks may occur due to inadequate conservation, cross-contamination, improper hygiene, and use of leftovers and illegal products (Lima et al., 2013, Da Cunha et al., 2016). A restaurant employee may wash vegetables to remove the dirt, bacteria, and/or pesticides that may have accumulated during cultivation or processing, but it has demonstrated that multiple washes with plain water may not significantly reduce the bacterial concentration on contaminated produce (Jensen et al., 2015). These infectious diseases are caused by the consumption of food or water that is contaminated with pathogenic microorganisms or toxins that are produced by them. Outbreaks are a global reality, and considered a public health problem of wide scope and have negative impacts on the productivity, economy and consumer confidence. According to Rahman et al. (2016), the Centers for Disease Control and Prevention (CDC) have reported numerous different foodborne pathogens that can cause infections in humans. From 2013 to 2015, several *Salmonella* outbreaks were reported worldwide, with one outbreak per year attributed to the consumption of cucumbers contaminated with this pathogen in the USA (CDC, 2013; Angelo et al., 2015; CDC, 2015).

The microbiological quality of vegetables that are eaten raw is a relevant factor to health that should be controlled and should be guaranteed by sanitization with chemicals that are effective in inactivating contaminant microbiota (Poimenidou et al., 2016). Vegetables provided in restaurants or as minimally processed are often linked to the origin of disease outbreaks because they are ready for consumption and are not subjected to sufficient processes to reduce microbial contamination. Thus, the use of effective chemical agents during the washing and sanitizing steps to ensure the safety of the products has gained more interest in the scientific community (Petri et al., 2015). A washing operation associated with the use of sanitizing solutions is considered the only process that can effectively reduce the number of spoilage-inducing and pathogenic microorganisms and contribute to the safety of the product (São José et al., 2014). The chemicals most often used to sanitize vegetables are chlorinated compounds (Rosário et al., 2017). Chlorine and its diverse forms are the most frequently used disinfectants. Easily use, low cost, good antimicrobial activity and dissolution in water make chlorinated agents attractive for frequent use in sanitization step in industry and restaurants (Petri et al., 2015). The recommended total chlorine concentrations as a disinfectant agent range from 50 to 200 $mg \cdot L^{-1}$ (Ali et al., 2017) In Brazil, it is recommended at a concentration of 100 to 250 $mg \cdot L^{-1}$ for 15 min for the disinfection of vegetables (Oliveira et al., 2012). Thus, the aim of this study was to survey and evaluate the procedures used in the sanitization step routinely applied for tomatoes in commercial restaurants in Vitória, Espírito Santo, Brazil.

MATERIALS AND METHODS

Experimental design

The first stage of the study was to survey the major sanitizing procedures of vegetables that are used in commercial restaurants located in Vitória, Espírito Santo, Brazil. The restaurants were contacted by an invitation letter that presented the research objectives and requested permission to visit. The responsible parties of each participating restaurant signed an authorization form to allow the research. For the sample definition, the total number of registered restaurants was defined as the number of restaurants in the Bars and Restaurants Union of Espírito Santo located in three districts which were selected for their proximity to the research institution. In 2014, 58 total commercial restaurants were registered and a final sample of 18 establishments was determined to be necessary to have a sampling error of 10% with a 90% confidence level.

The second stage of the study was conducted in a completely randomized manner, with each treatment subjected to three repetitions.

Survey of sanitization procedures

A checklist of sanitization procedures used by a previously trained researcher was used. The seventeen items in the list included the type of service, number of meals offered, sanitizers products used, duration of exposure, concentration of the food subjected to sanitization, containers used, training of the handlers to perform sanitation, presence of industry-specific tasks for pre-prepared salads, and use of instructional materials with guidance on how to sanitize properly and with which the protocol was developed.

Evaluation of sanitization protocols on natural contaminant microbiota

In the second phase of the study, the major sanitization methods used by the restaurants were used to analyse their efficiency to eliminate or reduce microorganisms. The sanitizers were sodium dichloroisocyanurate at a concentration of 200 $mg \cdot L^{-1}$ (NippoClor, Nippon Chemical®, São Paulo, São Paulo, Brazil), sodium hypochlorite at 200 $mg \cdot L^{-1}$ (Hidrosteril®, Itapevi, São Paulo, Brazil), 2% red vinegar (Toscano®, Várzea Paulista, São Paulo, Brazil), 2% acetic acid (Fmaia®, Belo Horizonte, Brazil) and running water. Acetic acid was studied as a sanitizing proposal, as it is currently observed with an interest in applying it for the sanitization of vegetables. For each treatment, approximately 250 g of tomato (*Solanum lycopersicum* L.) was immersed in one litre of sanitizing solution for 15 min. Tomatoes were acquired from local retailers and from a single producer to avoid variation. Tomatoes were stored under refrigeration at 7°C for a maximum of 24 h before processing, and damaged or rotten tomatoes were discarded. The tomato was chosen as a model system; it is widely consumed by the population in households and in commercials restaurants.

These sanitization methods were tested for their efficiency in reducing the count of natural contaminant microbiota (aerobic mesophiles, yeasts and moulds). Samples sanitized were subsequently subjected to microbiological analysis (Downes and

Ito, 2001). Samples of tomatoes were homogenized with 0.1% peptone water in a stomacher (Seward Medical Co., London, United Kingdom) for 2 min at normal speed. Appropriate decimal dilutions were prepared, and aliquots were transferred to growth media specific for the detection of each microbial group. To determine the number of aerobic mesophiles, inoculation was performed on standard agar plates for counting (Himedia®, São Paulo, Brazil) followed by incubation for 48 h at 35 ± 1°C. Yeasts and moulds aliquots were inoculated on potato dextrose agar (Himedia®, Brazil) acidified with 10% tartaric acid and incubated at 25 ± 2°C for 5 to 7 days. Plating rate experiments were performed in duplicate, and the results were expressed in colony-forming units per gram ($CFU \cdot g^{-1}$).

Removal of *Salmonella* Enteritidis ATCC 13076 cells attached to the surface of tomatoes

S. Enteritidis ATCC 13076 was obtained from stock culture. The culture was kept in 1 mL microtubes containing Brain Heart Infusion (BHI) broth (Himedia®, Brazil) with activation by two consecutive replications and incubated at 37°C for 18-24 h until the concentration reached 10^6 to 10^7 $CFU \cdot mL^{-1}$.

Tomato samples were selected and then cleaned and washed in sterile distilled water in aseptic conditions. After this, 250 g of tomatoes were placed in previously sterilized plastic bags. For each treatment evaluated, were used six plastic bags to place the tomatoes separately. After this, the inoculum (10 mL) was added with 100 mL of 0.1% of peptone water in each plastic bag. The plastic bag containing the inoculum and vegetables was lightly stirred for 5 min. The tomatoes were kept in static contact with the cell suspension for 60 min at 24 ± 1°C. Then, the cell suspension was drained, and the tomatoes that were contaminated with *S.* Enteritidis were placed in sterile plastic bags and incubated at 25°C for 24 h to allow bacterial adhesion.

Subsequently, the contaminated samples were subjected to the previously selected sanitization methods. As a control, inoculation without subsequent sanitization was performed. For each treatment, approximately 250 g of tomato were immersed in a litre of sanitizing solution for 15 min. After each treatment, 25 g of the tomatoes were transferred to sterile plastic bags containing 0.1% peptone water and then manually homogenized for 2 min. Then, 1 mL samples were removed to prepare serial dilutions that were plated by the surface spreading technique on *Salmonella Shigella* (Acumedia®, Indaiatuba, Brazil) agar. After incubation for 18 to 24 h at 37°C, colonies were counted (and recorded as $CFU \cdot g^{-1}$). To evaluate the effect of the sanitizing treatment, the units were converted from $CFU \cdot g^{-1}$ to log $CFU \cdot g^{-1}$. Counts from inoculated tomatoes that were not sanitized were considered as the initial count. The effect of the sanitizing treatments was calculated according to the following formula: exponential reduction = log (initial count with no sanitization) – log (final count after treatment).

Analysis of the sanitizing effect of removing *S.* Enteritidis adhered to the surface of tomatoes by scanning electron microscopy

For this analysis, we chose the best and worst treatment applied in the previous step. Thus, evaluations were made of tomatoes cuts treated with 2% acetic acid, and 2% red vinegar and samples that did not undergo sanitization. Samples of tomato were selected and then cleaned and washed in sterile distilled water. After washing the tomatoes, the outermost layer of the fruits was aseptically removed, and 1.0 cm sections were cut with the aid of a sterile scalpel. The sections were placed in Petri dishes containing sterile water for rinsing and removing waste from the plant tissue.

S. Enteritidis cells were grown in BHI broth (Acumedia® or Himedia, Brazil) for 16 h at 37°C. After this step, the broth was distributed onto the 13.5 cm diameter Petri dishes containing the tomato cuts. The cuts (n = 10) were then distributed into sterile plastic bags containing sterile distilled water for 1 min to remove planktonic cells and then subjected to the previously described sanitization methods. After sanitization, the sections were subjected to the microscopy preparation protocol.

The tomato cuts that were selected for observation in a scanning electron microscope were rinsed in phosphate buffered saline (PBS, 0.05 mol L^{-1}, pH 6.8 to 7.2) for removal of sanitizer residues and non-adherent cells. The fixation step consisted of a treatment in 5% glutaraldehyde in 0.1 M PBS buffer (v/v) for 1 h (25°C). The sections were then washed six times for 10 min in 0.05 M PBS buffer (pH 6.8 to 7.2). The dehydration step consisted of serial treatments in ethanol with 30, 50, 70, 80 and 95% ethanol for 10 min each and then three treatments of 100% ethanol for 15 min each. The samples were then transferred to a critical point drier (Critical Point Dryer – model CPD020, Balzers, Liechstenstein) for total dehydration. The samples were finally sputter coated (Denton Vacuum Desk II Sputtering, Denton Vacuum, Cherry Hill, N), and images were recorded using a scanning electron microscope, model JEOL JSM-6010LA (Jeol USA, Peabody, MA, USA). The analyses were performed in the Ultrastructure Cell Laboratory Carlos Alberto Redins (LUCCAR) of the Federal University of Espírito Santo.

Data analysis

The information collected in the first stage of the study were compiled into a Microsoft Excel spreadsheet for descriptive analysis of the data. Data were analysed with Genes® (Minas Gerais, Brazil) using the analysis of variance (ANOVA) method on the average of the logarithms of the number of colony forming units per gram (log $CFU \cdot g^{-1}$); post-test analysis was performed with the Tukey test, with a p-value <0.05 determined to be statistically significant.

RESULTS AND DISCUSSION

Survey of sanitization procedures

Eighteen commercial restaurants were contacted, and twelve agreed to participate in the research. In the evaluated restaurants, 58.3% (n = 7) were self-service types and 41.7% (n = 5) were a la carte, serving approximately 500 meals/day. All restaurants performed some hygiene procedure for vegetables. Washing and sanitizing fruits and vegetables are essential to prevent foodborne diseases (Petri et al., 2015). In this study, 91.66% (n = 11) of the restaurants use chlorinated compounds for their sanitization step, using one of four different brands of chlorinated compounds and sanitary water with the addition of 2.5% of sodium hypochlorite. Among the sanitizers used in the food industry and restaurants, especially to wash fresh produce, chlorine and chlorinated compounds are often used (Rosário et al., 2017). Their ease of use, low cost, high antimicrobial activity and complete dissolution in water make chlorinated agents a common choice for a disinfectant in

Table 1. Sanitation procedures adopted in restaurants in Vitória-ES, 2014.

Variables related of sanitation procedures	Yes (%)
Volume of sanitizing solutions	0.00
Technical manager	33.33
Sanitation	100.00
Use of registered product	83.34
Temperature control	0.00
Product reuse	0.00
Rinse after application of the product	83.34
Responsible and trained employee to carry out the procedure	0.00
Use of exclusive container for sanitation	100.00
Existence of instructional material to carry out the procedure	25.00
Existence of exclusive sanitation area	92.66

the fruit and vegetable industry (Petri et al., 2015).

In only one of the restaurants, a vinegar-based solution was followed by washing with water. Nascimento and Silva (2010) observed that the vinegar solution had a 50% lower reduction in the microbial load of the plant as compared to what was obtained with sodium hypochlorite. It is worth noting that all establishments surveyed in this study conducted sanitization procedures (Table 1), demonstrating the concern for this contaminant reduction step.

As for the contact time with the sanitizing solution, 50% of the restaurants that were surveyed immersed the vegetables for 15 min. In the others establishments, there was no controlled soaking time because the vegetables were left immersed during the period in which the handlers performed another activity. The time that food stays in contact with the sanitizing solution is well established (Chen and Zhu, 2011) as the samples are immersed in a sanitizing solution for approximately 15 min.

Regarding the concentration of chlorinated products used in the commercial restaurants, it was observed that all establishments used dilution metres and followed the manufacturer's recommendation. Oliveira et al. (2012) noted that 88% of visited restaurants did not use the sanitizer in pre-defined concentration. The concentration of sanitizer must be strictly controlled because it may lead to unacceptable sensory impact on the food. There was no temperature control for sanitizing in all the evaluated establishments. The best activity of chlorinated compounds is at a pH range between 6.0 and 7.5 and at low temperature (Banach et al., 2015). All commercial products used at participating restaurants were approved by the Ministry of Health, under the Brazilian regulations that are described in the DRC 216/2004. The sanitizing products should be identified and stored in a place reserved for this purpose (Brazil, 2004).

Regarding the volume of sanitizing solutions and the quantities of food sanitized at a time, there were no pre-established values in the surveyed establishments (Table 1). The use of large amounts of food in a low volume of sanitizing solution may cause a reduction in antimicrobial efficiency. Products that are used for sanitization must be applied properly in order to avoid residues on prepared food (Brazil, 2004; Oliveira et al., 2012).

All establishments that were surveyed use unique containers for the sanitization of vegetables, which corroborates with Oliveira et al. (2012), which found that most studied restaurants used unique tools for sanitization. It was observed that in 33.3% of the studied restaurants, the protocols were drafted by a technical manager of the establishment, a nutritionist.

In the establishments that were surveyed, only 75% were not observed in the presence of posters and instructional materials related to the execution of vegetable sanitization procedure near the area of pre-preparation. The presence of these materials facilitates the understanding of the manipulator, clarifying any doubts that arise during the execution of their functions in the pre-prepared vegetable area.

Efficiency of sanitization treatments on natural contaminant microbiota

After sanitization, a reduction in mesophilic aerobic count with all treatments was observed, with the greatest reduction occurring after treatment with acetic acid 2% ($p < 0.05$) (Table 2). There was no significant difference in the score between tomatoes without sanitization and tomatoes immersed in running water ($p > 0.05$). This result demonstrated the importance of the application of sanitizing compounds to inactivate microorganisms and guarantee food safety. Regarding the aerobic mesophilic count, Brazilian legislation (Brazil, 2004) does not provide a limit to the maximum count allowed on fresh vegetables, so the maximum count was considered based on the recommendation of a maximum value of

Table 2. Effect of sanitizing treatments for 15 min on reduction of natural microbiota on tomatoes (*Solanum lycopersicum* L).

Treatments	Aerobic mesophiles (Log CFU·g^{-1})	Reduction (Log CFU·g^{-1})	Mould and yeasts (Log CFU·g^{-1})	Reduction (Log CFU·g^{-1})
No sanitizer	$4.82^{a} \pm 0.44$	-	$5.02^{a} \pm 0.22$	-
Running water	$4.50^{a} \pm 0.45$	0.32	$4.34^{ab} \pm 0.18$	0.68
Sodium dichloroisocyanurate 200 mg·L^{-1}	$4.08^{ab} \pm 0.88$	0.74	$4.08^{ab} \pm 0.94$	0.94
Sodium hypochlorite 200 mg·L^{-1}	$3.41^{ab} \pm 0.18$	1.09	$3.81^{ab} \pm 0.72$	1.21
2% acetic acid	$2.93^{c} \pm 0.20$	1.86	$3.23^{b} \pm 0.25$	1.79
2% red vinegar	$3.31^{bc} \pm 0.31$	1.51	$3.76^{ab} \pm 0.52$	1.26

*The values presented are means followed by standard deviation (mean ±SD). Means marked with same letter in the same column do not differ ($p > 0.05$) between themselves.

10^5 to 10^6 CFU·g^{-1} (Morton, 2001). Comparing the results shown in Table 1 with this limit, after sanitization, all the tomatoes were suitable for consumption. Fantuzzi et al. (2004) obtained similar results when assessing the immersion of cabbage in sanitizing solutions and verified a significant decrease of up to 1.8 log CFU·g^{-1} of mesophilic aerobic bacteria as compared to samples that were washed only in water.

Oliveira et al. (2012) found different levels of effectiveness of the sanitization processes in lettuce than was found in this study with tomatoes; specifically, a 200 mg L^{-1} hypochlorite solution with 30 min of exposure promoted a 2.5 log CFU·g^{-1} reduction in bacteria. These results promoted better reduction probably because of the higher time of contact and the particular features of the surface of the sanitized vegetable that are studied. According to Yuk et al. (2006), the microstructures of the plant tissue, such as gouges, cracks, cavities and other irregularities of the surface of the vegetable, can alter the contact of the sanitizing solution with the microorganisms and consequently affect the sanitization efficiency.

After sanitization, a decrease in moulds and yeasts was observed, with the greatest reduction occurring after treatment with 2% acetic acid ($p < 0.05$). However, Fantuzzi et al. (2004) found no significant difference in the reduction of microbial contaminants in cabbage samples treated with 1% acetic acid as compared to washing only with water. However, the present study showed that a higher concentration of acetic acid was more effective than water. Poimenidou et al. (2016) showed that vinegar was effective against *E. coli* O157:H7 with a 2.0 to 2.4 log CFU·g^{-1} reduction on spinach samples and a 1.8 to 2.3 log CFU·g^{-1} reduction on rinsed lettuce and vinegar-treated samples maintained the total viable cell counts at low levels during storage. The impact of vinegar on lettuce samples was not significant when the treatment was applied for only 2 min. Organic acid solutions and plant-derived compounds have gained attention due to their antimicrobial activity and their consumer-friendly nature. Organic acids are generally recognized as safe (GRAS) and their bactericidal efficacy against *E.coli* O157:H7, *Listeria monocytogenes* and *Salmonella* on fresh produce has been previously investigated (Huang and Chen, 2011; Sagong et al., 2011; Poimenidou et al., 2016). Nascimento and Silva (2010) treated strawberries with different chemical products and observed greater reductions with 4% acetic acid, specifically with reductions of 1.18 and 1.34 log CFU·g^{-1} for mesophilic aerobic and moulds and yeast. Park et al. (2011) observed that after 10 min of treatment in apples, 1 and 2% acid acetic promoted 0.52 to 2.78 log reduction and exhibited significant ($p < 0.05$) antibacterial effects against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* as compared to the control treatment.

There were no significant differences between the tomatoes treated with a solution of sodium dichloroisocyanurate and those treated with sodium hypochlorite ($p > 0.05$) for both groups of microorganisms. According to São José and Vanetti (2012), under the typical wash conditions of vegetables, the efficiency of chlorine compounds in reducing microbial contamination is limited, achieving a two-logarithmic reduction in the population of microorganisms. Thus, the results of treatment with 2% acetic acid, which promoted greater reduction in contaminant natural microbiota, is noteworthy. Additionally, it is worth noting that vinegar contains acetic acid, a commonly used ingredient in vegetable salads, and is considered an alternative sanitizing agent for the inactivation of pathogens (Sengun and Karapinar, 2005). However, the concentration of acetic acid in vinegar solutions is low, which may contribute to their reduced efficiency in microbial inactivation.

Evaluation of sanitization treatments on *S.* Enteritidis ATCC 13076 cells attached to the surface of tomatoes

After sanitization with 2% acetic acid, a reduction in *S.* Enteritidis counts was observed, and this reduction was

Table 3. Effect of sanitizing treatments for 15 min to inactivate *S.* Enteritidis ATCC 13076 cells intentionally inoculated in tomatoes (*Solanum lycopersicum* L).

Treatments	*S.* Enteritidis (Log CFU·g^{-1})	Reduction (Log CFU·g^{-1})
No sanitizer	6.11^{a} ± 0.43	-
Running water	5.55^{ab} ± 0.56	0.55
Sodium dichloroisocyanurate 200 mg·L^{-1}	4.87^{ab} ± 0.10	1.23
Sodium hypochlorite 200 mg·L^{-1}	4.84^{ab} ± 0.57	1.26
2% acetic acid	4.07^{b} ± 0.83	2.04
2% red vinegar	5.63^{a} ± 0.54	0.47

*The values presented are means followed by standard deviation (Mean ±SD). Means that are marked with the same letter in the same column do not differ (p > 0.05) between themselves.

significantly higher than the other methods that were applied (p < 0.05) (Table 3). In the study by São José et al. (2014), green peppers sanitized with 1% acetic acid for 2 min resulted in a reduction of 1.6 log CFU·g^{-1}. According to Nastou et al. (2012), the efficiency of acetic acid can be limited and vary with the treated vegetable. There was no significant difference between treatments with chlorine compounds and running water (p> 0.05); both treatments did not show satisfactory results in the reduction of *Salmonella* cells that were adhered to the surface of the tomato. The discussion on the use of chlorinated compounds is related to the possibility of generating highly carcinogenic by-products such as trihalomethanes, trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane (São José and Vanetti, 2012). This reinforces the need to apply appropriate methods of sanitization to fruits and vegetables.

Yang et al. (2009) evaluated the inactivation of *L. monocytogenes, Escherichia coli* O157:H7, and *Salmonella* Typhimurium with compounds available in households and observed that after 1 min at 25°C, 3% hydrogen peroxide achieved a >5 log CFU·g^{-1} reduction of both *S.* Typhimurium and *E. coli* O157:H7, whereas undiluted vinegar had a similar effect only against *S.* Typhimurium. In a study by Sengun and Karapinar (2005), it was observed that 30 min of treatment with 50% vinegar on rocket leaves resulted in a reduction of 2.81 log CFU·g^{-1}. In the same study, scallion samples treated for 60 min had a reduction of 2.1 log CFU·g^{-1} of the initial population of *Salmonella*. According to Machado et al. (2012), different types of microorganisms may have varying responses to action of antiseptics and disinfectants.

Analysis of the effect of sanitizers on the removal of *Salmonella* adhered to the surface of tomatoes by scanning electron microscopy

The images confirm bacterial adhesion to the surface of tomatoes without sanitization (Figure 1). The fact that *Salmonella* grows and forms biofilms on the surface of tomatoes and other foods can hinder the action of sanitizers. The ability to strongly adhere to the plant epidermis may reduce the efficiency of the decontamination treatments and complete microbial inactivation might not be possible (Costa et al., 2012). In image B, the removal of attached *Salmonella* cells after treatment with 2% vinegar can be observed. In image C, it can be seen that 2% acetic acid promoted considerable removal of surface-adherent cells, and the tomato had a neater appearance than the other treatments. The inability of sanitizers to remove all microorganisms from the tomato surface suggests a potential for microbial growth in the event of post-sanitization storage and also suggests a chance that pathogenic cells remain on parts of the plant surface or as pre-existing biofilms.

Most of the results presented in the literature are of studies carried out evaluating treatments applied at industrial level but present compatible results. It is known that sanitizing treatments with chlorinated compounds are applied in both food industry and food services. However, caution should be applied on sanitizer application to avoid chemical residues generation that can influence the flavor and aroma of vegetables.

Conclusion

All the studied establishments sanitized vegetables with solutions based on chlorinated compounds or vinegar. These treatments promoted an average reduction of 1 log CFU•g-1, less than the proposed treatment of 2% acetic acid, which was more effective in both reducing contaminants in natural microbiota as well as in tomatoes inoculated with S. Enteritidis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

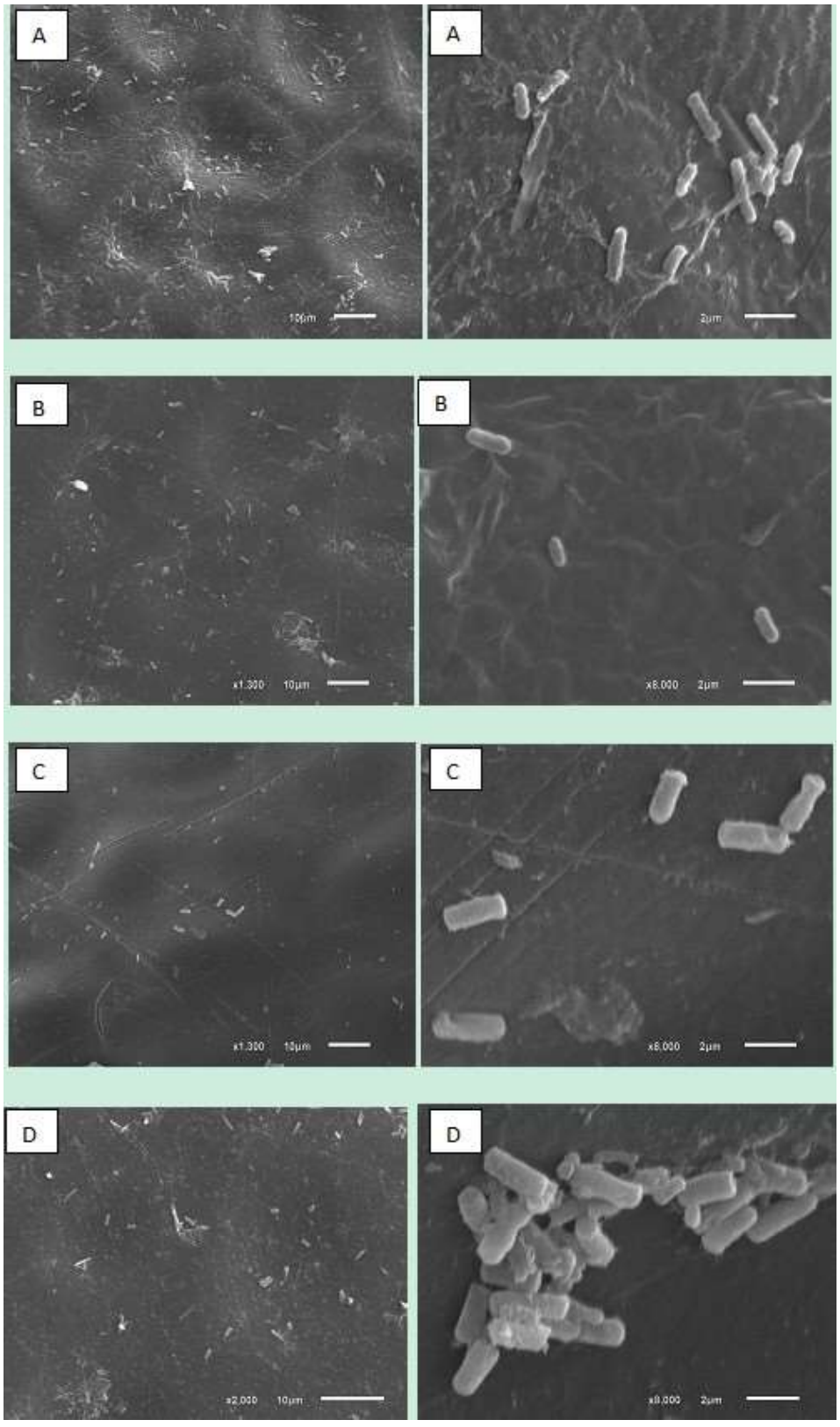

Figure 1. Images obtained by scanning electron microscopy. Photomicrographs of tomatoes cuts intentionally contaminated with *S.* Enteritidis: A) no sanitizing, B) treatment with 2% vinegar, C) treatment with 2% acetic acid, D) dichloroisocyanurate sodium 200 mg/L.

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Vol. 11(46), pp. 1657-1662, 14 December, 2017
DOI: 10.5897/AJMR2017.8719
Article Number: 83043E267087
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

A retrospective study of antibiotic sensitivity pattern of uropathogens in the Federal Capital Territory, Nigeria

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Received 25 September, 2017; Accepted 30 November, 2017

This study was designed to bring to the fore the prevalent causative agents of urinary tract infections (UTIs) and their antibiotic susceptibility patterns in Nigeria, using patients attending University of Abuja Teaching Hospital (UATH) as a case study. Using microbiological/biochemical methods, prevalence of uropathogens amongst sexes, was compared between 2010 and 2015. Bacterial counts (10^5/ml) in the urine was used as quantitative standard and the total number of patients in the study was 214 (166 females and 48 males). The prevalence of UTIs in samples collected from 214 patients between 2010 and 2015 shows that *Escherichia coli* (57.5%) was the most prevalent organism causing UTIs, followed by *Klebsiella* species (18.5%), *Staphylococcus aureus* (11.2%) and *Proteus* species (12.4%). Antibiotics used include nitrofurantoin, ofloxacin, nalidixic acid, amoxicillin, gentamicin, tetracycline, levofloxacin, and augumentin, with bacterial causing UTI displaying greatest resistance to tetracycline and nitrofurantoin the highest sensitivity. This study indicates most of the uropathogens are still susceptible to antibiotics commonly used in the hospital. However, *E. coli* exhibited resistance to amoxicillin. The development of antimicrobials for prevention and treatment of infections should be tackled from a worldwide understanding of infection patterns so as to overcome the increasing level of superbugs in general and UTIs in particular.

Key words: Urinary tract infections, microbial resistance, adaptive immune response.

INTRODUCTION

Urinary tract infection (UTI) refers to the presence of bacteria (>10^5 bacteria per ml urine) in the urinary tract together with symptoms and sometimes signs of inflammation. UTI is one of the most commonly occurring bacterial infections among men and women (Liza and Jonathan, 2006). Due to the frequency of UTI, it necessitates more than 1.0 million hospital admissions with high economic burdens, which is estimated at $1.0 Billion U. S. Dollars (USD) of global healthcare expenditures (Foxman, 2003; Schappert and Rechtsteiner, 2007).

The prevalence of UTI varies markedly as the infection

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is problematic to all age groups. Microorganisms can reach the urinary tract by haematogenous (Daoud and Afif, 2011; Servin, 2014) or lymphatic spread (Kaper et al., 2004) via interaction between bacteria virulence and the host. Approximately 60% of all women will have a UTI during their lifetime (Daoud and Afif, 2011; Foxman, 2003).

UTIs also known as cystitis (bladder infection) when it affects the lower urinary tract and pyelonephritis (kidney infection) when it affects the upper urinary tract. In the lower urinary tract, it is characterized by burning sensation with either frequent urination or urge to urinate or both with significant pain (Nicole, 2008), although these symptoms may vary from mild to severe (Lane and Takhar, 2011; Chen et al., 2013). In healthy women, the pain lasts an average of six days (Colgan and Williams, 2011). However, in the upper urinary tract, it is characterized by flank pain, fever or nausea (Lane and Takhar, 2011; Chen et al., 2013). The most predominant etiologic agent of UTI is the *Escherichia coli* causing about 80 to 85% of the cases of UTIs, with *Staphylococcus* being the cause in 5 to 10% (Chen et al., 2013; Nicole, 2008). The prevalence of UTIs in women may be due to the proximity of the urethra to the anus (Aboderi et al., 2009; DeBacker et al., 2008). Moreover, as a woman's oestrogen hormonal level decreases due to the onset of menopause, the risk increases due to the loss of protective innate flora. In both sexes, any condition (as in the cases with diabetes, spinal cord injuries and in HIV-positive individuals) that reduces the efficacy of bladder emptying or irritates the urinary tract can cause UTIs (Samuel et al., 2012).

In Nigeria, symptomatic patients usually indulge in indiscriminate usage of antibiotics before consulting a physician. The physicians also, usually treat patients with different antibiotics without any substantive investigation (Abdorin et al., 2009). Resistance to antibiotic by bacterial and other super-bugs is an emerging and serious health problem resulting in increased morbidity and mortality (Croxen et al., 2013). In the UK alone, more than 5,500 people died from *E. coli* infections, and many of them were due to strains resistance to antibiotics (news.sky.com, 2016). The underlying molecular mechanisms for bacterial resistance to antibiotics have not been fully studied, although they are thought to include processes such as enzyme-catalyzed antibiotic modifications, bypass of antibiotic targets and active efflux of drugs from the cell (Wright, 2011; Croxen et al., 2013). Moreover, such resistance may/could also be propagated via enhanced horizontal or lateral gene transfer (LGT). LGTs can induce harmful mutations, and this can cause bacteria to resist antibiotics, creating different strains of bacteria with varying degrees of resistance due to genetic mutation (Robinson and Hotopp, 2016). UTI resistance rates against commonly prescribed antibiotics are constantly rising. For instance, it is noted that up to 20% of uropathogens are resistant to Trimethoprim/Sulfamethoxazole (TMP/SMX) and Cepholosporins. This increasing resistance is also being observed with the use of Fluoroquinolones, with resistance rates rising up to 10% (DeBacker et al., 2008).

This study was designed to evaluate UTIs and the sensitivity patterns of etiologic agents. The prevalence of UTI infections was determined using patient's bio-data (age and sex) obtained from the University of Abuja Teaching Hospital in Gwagwalada, Abuja. Also, the antibiotics susceptibility pattern to uropathogen isolates was determined.

MATERIALS AND METHODS

Aseptic collection of urine specimens

Patients collected their midstream urine in sterile bottles, closed tightly and brought to the laboratory. In the laboratory, the urine was physically analysed based on its turbidity or clearness. Bacterial counts (10^5/ml) in the urine of the patient were used as the quantitative standard of bacterial counts in the samples.

Media used

The used media included CLED agar, chocolate agar, nutrient agar for sensitivity test, peptone broth, triple sugar iron agar and Simmon's citrate agar. Each isolate was Gram-stained and subjected to biochemical tests to identify the microorganism using standard biochemical tests (Ho et al., 2004). Aseptic techniques were utilized in each of the tests.

Antibiotics

Antibiotic sensitivity test (AST) was used to determine the antibiotic that would be most successful in treating a bacterial infection using antibiotic disks (Bauer et al., 1966). The diameters of the zones of clearing around each antibiotic disk were measured in millimetres to determine the sensitivity of the isolates to the antibiotic (Bauer et al., 1966).

Quantitative analysis

Using the spread plate, urine samples were directly inoculated by streaking on the media, then incubated for 24 h at 37°C to check microbial growth.

Culture observation

Colour, size and colony morphology were observed from cultured plates. Each isolate was subjected to Gram-staining and their Gram's reaction was recorded as positive or negative.

Biochemical tests

The biochemical tests carried out for identification of the organisms were according to standard microbiological and biochemical techniques, and these tests were namely catalase, coagulase, Simmons' Citrate Test, Urea Agar Base, and Triple Sugar Iron Agar Test (Ho et al., 2004; Nwachukwu et al., 2014).

Table 1. Prevalence of uropathogens from males and females from 2010 to 2015.

Uropathogens	Prevalence (%)											
	2010		2011		2012		2013		2014		2015	
	Male	Female	Male	Female	Males	Female	Males	Female	Male	Female	Male	Female
Escherichia coli	10 (36)	18 (64)	4 (33)	8 (67)	10 (42)	14 (58)	8 (30)	19 (70)	2 (18)	9 (82)	5 (16)	27 (84)
Klebsiella spp.	2 (33)	4 (67)	2 (25)	6 (75)	1 (11)	8 (89)	0 (0)	7 (100)	0 (0)	4 (100)	1 (11)	8 (89)
S. aureus	1 (25)	3 (75)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	5 (100)	0 (0)	2 (100)	2 (18)	9 (82)
Proteus spp.	0 (0)	1 (100)	1 (33)	2 (67)	1 (33)	2 (67)	2 (22)	7 (78)	1 (50)	1 (50)	3 (27)	8 (73)

RESULTS

The prevalence of UTIs in samples collected from 214 patients between 2010 and 2015 treated at the University of Abuja Teaching Hospital of which males and females accounted for 48 (22.4%) and 166 (77.6%) of this number, respectively, are presented within this study.

Table 1 shows the total prevalence of uropathogens in both male and female patients from 2010 to June 2015 and it also indicates the mean percentage prevalence of uropathogen isolates according to gender in the years under review.

Figure 1 shows the average percentage sensitivity of the isolate to the most sensitive antibiotics tested against the various bacterial causing UTI. These antibiotics across board indicate over a 50% efficacy with *E. coli*, being the most prevalent causes of UTI, showing above 80% response as against other antibiotics, except for gentamicin, presented within this study. *Staphylococcus aureus*, however, exhibited above 80% sensitivity response to only Levofloxacin and Nalidixic acid; while, *Klebsiella* and *Proteus* species exhibited similar response to Oflaxacin and Levofloxacin. The average resistant of isolates to antibiotics as revealed from the study are as shown in Figure 2 with all the UTI isolates, which demonstrated a higher than 50% resistance. *Klebsiella* spp. exhibited a higher than 80% resistance to the antibiotics used (Figure 2), whilst *Proteus* spp. showed a 60% resistance and above, except for Cotrimoxazole; *E. coli* percent resistance was also 60% and above, except for Augmentin; while *S. aureus* was 60% and above across all the antibiotics utilized within this study.

DISCUSSION

UTIs are one of the most common infections encountered in the population with about 150 million infections estimated per year worldwide (Sharef et al., 2015). The results of the current work, indicates a number of uropathogens causing UTI including *E. coli*, *Klebsiella* spp., *Proteus*, *S. aureus* and *Candida albicans*. According to the results of this study, *E. coli* happens to be the most prevalent causative agent in all age groups and both sexes during the years are covered. *S. aureus* is a facultative anaerobic Gram-positive cocci, and it is also prevalent among patients with UTI. *S. aureus* is a commensal organism in the peri-anal and vaginal regions; therefore, emphasis on personal hygiene, most especially amongst females, may be important in reducing the UTI occurrences (O'Brien et al., 2015).

Results from this study indicates that females are the most susceptible to UTI, and this may be due to a shorter and wider urethra, which is more transversed by microorganisms, and the retrograde ascent of bacteria from the faecal flora via the urethra to the bladder and kidney (Kolawale et al., 2009). This result trend had also been exhibited in other studies previously carried out in Nigeria (Kolawale et al., 2009; Mbata et al., 2007).

The results indicate that ofloxacin, nitrofurantoin, nalidixic acid and levofloxacin have higher antimicrobial activity against most isolates (Figure 1) as compared to augumentin, tetracycline and amoxycilin. The high rate of bacterial resistance to the latter antibiotics may be due to the fact that they are most commonly prescribed thus their misuse may have helped conferred some form of resistance by UTI causing bacterial.

In UTI reoccurrence, the role of adaptive immune responses (AIR) has not been fully studied; hence their responses are not well understood. However, adaptive immune responses appear to contribute to immunity

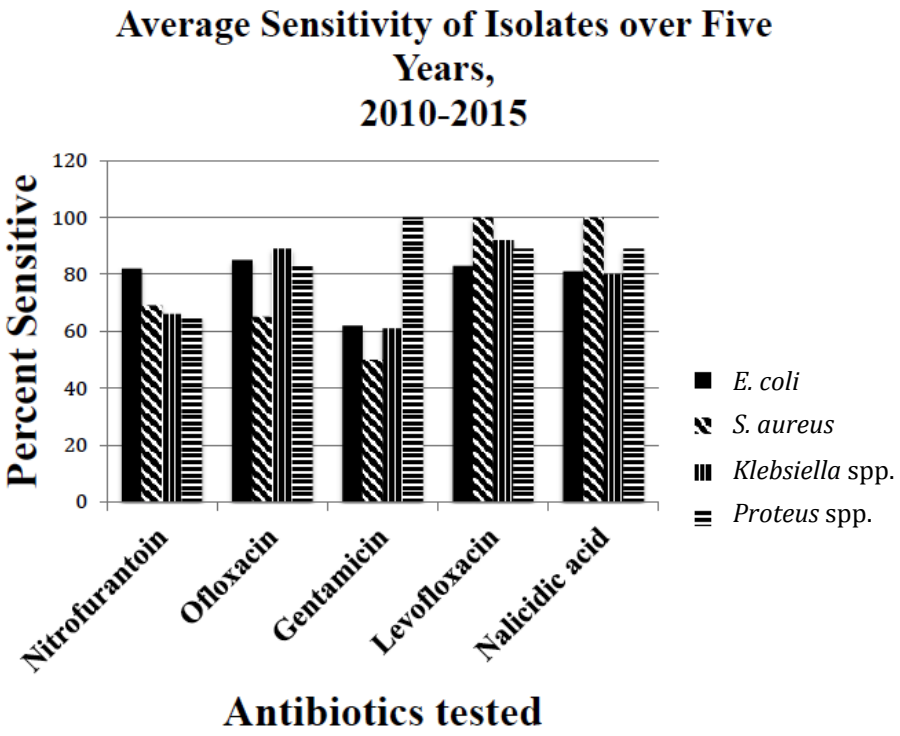

Figure 1. Average sensitivity of isolates over five years 2010-2015.

defence against UTI challenges (Thumbika et al., 2006).

This perhaps explains why some women who suffer an acute UTI do not necessarily develop a recurrent infection (O'Brien et al., 2015). Moreover, non-orthodox innate immune response to UTIs has also been established as, for instance, a novel role for yersiniabactin in UTI, which has been recently identified (Chaturvedi et al., 2013). Yersiniabactin is a siderophore, in other words a small molecule that scavenges and imports free iron, and was found to have superoxide dismutase (SOD)-like activity, hence preventing bacterial mortality in phagocytic cells that are depleted of copper and/or iron (Chaturvedi et al., 2013). To counter the effects of bacterial scavenging by transition metals, the host produces the antimicrobial protein lipocalin-2, which binds and inactivates siderophores such as yersiniabactin. It been noted that, during the onset of cystitis, lipocalin-2 protein expression is induced in the bladder epithelium (Duell et al., 2012; Taneja et al., 2008). Further, α-intercalated cells of the collecting duct present in the kidney were found to act as a molecular sieve of the upper urinary tract during cystitis, once they sensed an infection in the lower urinary tract, in a Toll-like Receptor 4 (TLR4)-dependent manner leading to the expression and secretion of lipocalin-2 into the urine filtrate (Paragas et al., 2014).

Moreover, the understanding for the role of some proteins/peptides, as defensive mechanisms against UTIs, are only becoming obvious. For instance, the protein beta defensin-1 (BD1) and Cathelicidin (LL-37) peptide shows constitutive expression in the urinary tract, as both molecules from previous studies have demonstrated to play key roles in UTI's mitigation (Mambula et al., 2000; Chromek et al., 2006). Also, in a study of uncomplicated UTI subject, elevated level of LL-37 was observed (Nielsen et al., 2014), whilst *E. coli* isolates from healthy controls exhibited more susceptibility to LL-37 than isolates from UTI patients. Further, BD1 has also exhibited constitutive expression in the urinary tract and hence, BD1 is suggested to play a role in pyelonephritis (Smith et al., 2011; Mambula et al., 2000; Morrison et al., 2002) and act as a defence against Gram-positive uropathogens (Morrison et al., 2002). Moreover, studies on defensins (Mambula et al., 2000), contained in neutrophil granules, indicated antifungal activity. These peptides combined are potential molecules of the immune system that could provide clues for the biochemical processes that can be modified to overcoming the increasing level of superbugs in general and bacterial resistance to antibiotic in UTI management in particular.

It is encouraging that the search for molecules of the immune system that could serve as alternatives to the growing resistance of some bacterial strains to antibiotics is on the increase, this is indicated in a recent study of peptides like clavin-MO (Silva et al., 2016), which have exhibited good results against strains of *E. coli* and *S. aureus*.

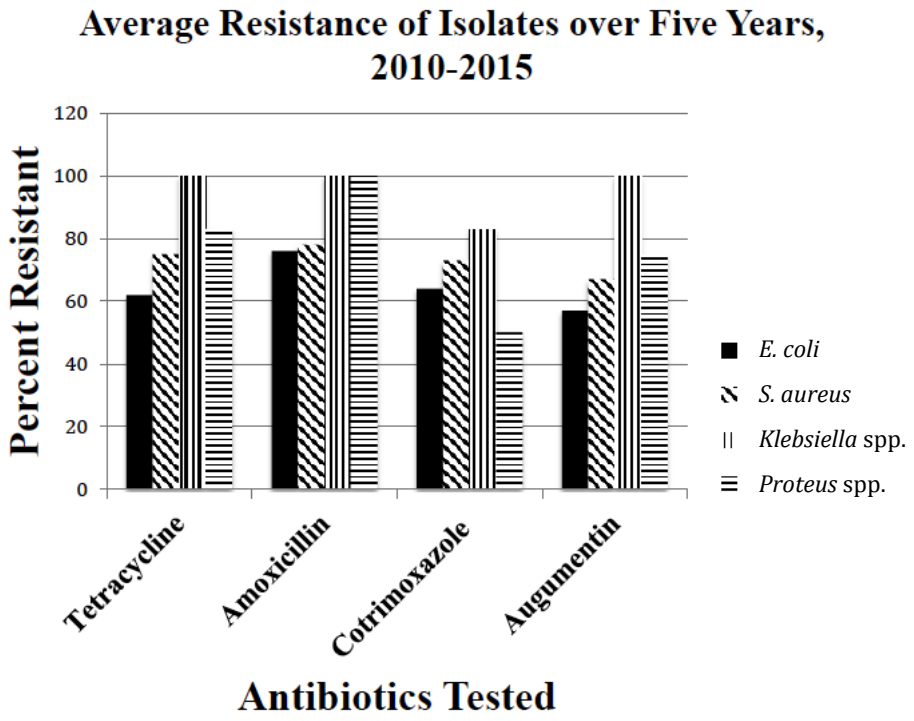

Figure 2. Average resistance of isolates over five years, 2010-2015.

From the foregone analysis, it is only reasonable that moving forward any mitigation process would require a holistic approach to include a requisite modern molecular biology tool-kit, such as Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated proteins 9 (CRISPR-Cas9), once such tool-kit has undergone the appropriate approval processes. CRISPR-Cas9 is a DNA-editing tool, which if deployed to target sequence specific moieties in the infectious bacteria, could help attenuate or inactivate production of the gene/protein conferring resistance to antibiotics. These adaptive immunity systems could in turn help to modify the host genome in the fashion of retaining the memory of past infections. Using these modern molecular tool-kits, we can only wish scientist and physicians from transition economies will be carried along, as such would enhance management of UTI and antimicrobial resistance in general.

This study indicates that most of the uropathogens causing UTIs are still susceptible to antibiotics commonly used in the hospital and community pharmacies. However, *E. coli* in particular exhibited resistance to amoxicillin and these results are similar to those of other recent studies (Kolawale et al., 2009; Tadesse et al., 2012).

Conclusions

Generally, UTI infections due to *E. coli* are thought to develop high antibiotic resistance. Thus, it is essential that effective antimicrobials for prevention and treatment of infections are developed to overcome the increasing level of superbugs in general. This is so important bearing globally; patients with infections caused by drug-resistant bacteria are at increased risk of death due to worse clinical outcome. This study was designed and aimed at bringing into focus the prevalent causative agents of UTIs and their antibiotic susceptibility patterns amongst patients attending UATH. Moving forward, it was proposed that the guideline for the approach to UTI management should include the requirement of identifying the causative organisms through urine culture and choosing the appropriate antibiotic through *in-vitro* sensitivity tests, thus down playing incessant/indiscriminate antibiotic usage. In addition, the study of antibiotic susceptibility patterns is very important for the development of empirical treatment guidelines for UTI management.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 11(46), pp. 1663-1668, 14 December, 2017
DOI: 10.5897/AJMR2017.8720
Article Number: CD3BE9167088
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Variability in the genotypes of rotavirus detected in Côte d'Ivoire from 2010-2016

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Received 27 September, 2017; Accepted 13 November, 2017

Group A rotaviruses are the major viral agent of acute gastroenteritis and severe diarrhea in children <5 years old. The World Health Organization (WHO) recommends surveillance of circulating strains before and after introduction of vaccination in countries. However, the diversity of circulating strains in developing countries is a major challenge to the vaccination programs. This study, carried out in furtherance of the sentinel surveillance, aims to identify the different genotypes circulating before the introduction of the Rotavirus vaccine. All children with acute gastroenteritis aged 0 to 5 years, admitted in one of the sentinel surveillance collection sites were included in the study. The study period was from January 2010 to December 2016. Rotavirus was detected in stool specimens by enzyme-linked immunosorbent assay (ELISA). Rotavirus G and P types were determined by real-time polymerase chain reaction (RT-PCR). A total of 1472 stool samples were collected during this period. 31.8% of the stools were rotavirus positive by ELISA test. G1 was predominant with 39.6% followed by G12 (27%). P [8] was 50.4%. The predominant genotype combinations were G1P [8] with 26.1%; G12P [8], 15%; G1P [6], 11.3% and G12P [6], 10.8%. Genotyping of circulating rotavirus strains is important in monitoring strains before and after the introduction of the vaccine. With previous observations, these findings will contribute to baseline data to further monitor the impact of rotavirus immunization in Côte d'Ivoire.

Key words: Rotavirus, Côte d'Ivoire, diarrhea, vaccination, acute gastroenteritis.

INTRODUCTION

Group A rotaviruses are the major viral agent of acute gastroenteritis and severe diarrhea in children <5 years old (Gasparinho et al., 2017). Globally, this virus is responsible for about 40% of cases of severe diarrhea with hospitalization and 5% of deaths in children under five years (Eteme et al., 2015). The number of

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deaths is estimated to be 215,000/year in children <5 years with 80% cases in sub-Saharan Africa and South Asia (Tate et al., 2016). The envelope of rotavirus is made of VP7 and VP4 proteins which form the outer part of the capsid (15 genotypes G and 27 genotypes P) (Lorrot et al., 2012). The circulating genotypes of rotavirus are of different types all over the world; several studies in Africa have identified the G1P [8] genotype as the most common in pre-vaccination areas. The G1P [6], G8P [6], G6P [6], G8P [8], G12P [6] and mixed G and mixed P genotypes are also found in a lower rate in sub-Saharan Africa (Hokoro et al., 2014). The World Health Organization (WHO) recommends surveillance of circulating strains before and after introduction of vaccination programs in various countries (Damanka et al., 2016). However, the diversity of circulating strains in developing countries proves a real challenge to the vaccination programs (Todd et al. 2010). Currently, WHO recommends two types of oral vaccines: the Rotarix® vaccine (GlaxoSmithKline, Rixensart, Belgium) and Rotateq® vaccine (Merck & Co., Whitehouse Station, NJ). Rotateq® vaccine contains (RV5) five human-bovine reassortant viruses [W179-9 (G1P [5], SC-2 (G2P [5]), W178-8 (G3P [5]), BrB-9 (G4P [5]) and W179-4 (G6P [8]). Rotarix® vaccine (RV1) is a human G1P [8] virus RIX4414 derived from a serial passage in the cell culture of a virus recovered from the stool of an infected child (Agutu et al., 2017). Both recommended vaccines require multiple dose administration (two doses for Rotarix and three for RotaTeq); the first to be administered between 6 and 15 weeks of age to raise homo- and heterotypic immune response against RVA different strains. The two vaccines have been proven to be effective worldwide, but lower efficacy was observed in low-income countries from Africa and Southern Asia. Among the several hypotheses used to explain the differences in the immune response and consequent efficacy of these vaccines in low- versus high-income countries, RVA strains diversity, host genetic factors, malnutrition, host co-infection, deficient micronutrient ingestion, and interfering gut flora have been put forward (Gasparinho et al., 2017). Studies conducted in Côte d'Ivoire prior to the sentinel surveillance which started in 2010 showed a predominance of G1 followed by G2. A small number of G3, G8 and G9 variants were identified in this study (Akran et al., 2010). In view of the wide variety of circulating strains, it is therefore important to maintain continuous monitoring of the prevalence of rotavirus in order to understand the distribution of G and P genotypes in the country. Accurate information in respect of different types of circulating genotypes of rotavirus is essential to monitor the impact and effectiveness of the vaccine.

This study aimed to identify the different genotypes circulating in the country before the introduction of the Rotavirus vaccine. It proposes to describe the proportion of the different genotypes G and P circulating from 2010 to 2016 in the pre-vaccination area. Genotypic combinations were also determined.

MATERIALS AND METHODS

Study population

Children with acute gastroenteritis aged 0 to 5 years, admitted in one of the sentinel surveillance sites were recruited. There are six collection sites altogether in five municipal localities in the city of Abidjan, the commercial capital of Côte d'Ivoire. Children either hospitalized or kept under observation for treatment of acute diarrhea (less than two weeks) were included in the study with stools that had no mucus or blood associated with fever from January 2010 to December 2016.

Stool samples

Stools samples were collected from each child after obtaining informed consent from the parents, in a sterile jar on the same day or the next day of admission. Participants were request to complete some forms containing details of the child's socio-demographics and clinical information. The samples were kept in refrigerator at a temperature of between 0 and 4°C, and sent to the laboratory at the Yopougon University Teaching Hospital, Bacteriology-Virology unit, where they were stored at 4°C for a maximum of 30 days until ELISA test was performed. ELISA positive samples were then stored at -20°C before being taken to one of the WHO rotavirus reference laboratories, namely the Limpopo Regional Laboratory in South Africa or the West Africa Regional Laboratory in Ghana for genotyping.

Laboratory analysis

Detection of group A Rotavirus antigens

Samples were screened for the presence of rotavirus structural protein VP6 by the use of Rotaclone® a rapid EIA test kit following the manufacturers' instructions. Samples with optical density >0.25 at 450 nm wavelength were considered positive.

Molecular characterization of Rotavirus strains

G- and P-genotyping assays

To determine the VP7 (G-) and VP4 (P-) genotypes, viral RNAs was extracted from the clarified supernatant of 20% stool suspensions using the QIAamp® Viral RNA Mini kit (QIAGEN®, Hilden, Germany) based on the manufacturer's instructions. Reverse transcription (RT)-PCR was performed using both forward and reverse consensus primers Beg9/End9 and Con3/Con2 to amplify a 1,069bp and 835bp fragments of the VP7 and VP4 genes respectively. Multiplex PCR was carried out for G- and P-typing with genotype specific primers as previously described (Gouvea, 1990; Gentsch, 1992; Iturriza-Gomara, 2004). PCR amplicons were electrophoresed on a 2% agarose gel in Trisborate- EDTA buffer together with a 100-bp DNA ladder.

Data analysis

All statistical analysis was performed with the EPI-Info version 3.5.4 software (CDC Atlanta, USA). All categorical variables were summarized as proportions, and significance of their difference in distribution with the outcome was assessed using Pearson's Chi-square and Fisher test at 5% risk.

Table 1. Sex distribution and ELISA test results, 2010-2016.

Characteristic	Year							
	2010	2011	2012	2013	2014	2015	2016	Total
Sex	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Female	41 (43.2)	26 (50)	83 (36.4)	131 (41.8)	89 (40.1)	153 (47)	104 (43.9)	627 (42.6)
Male	54 (56.8)	26 (50)	145 (63.6)	182 (58.2)	133 (59.9)	172 (53)	133 (56.1)	845 (57.4)
Total	95 (100)	52 (100)	228 (100)	313 (100)	222 (100)	325(100)	237 (100)	1472 (100)
ELISA test result								
Negative	76 (80)	34(65.4)	169 (74.1)	219 (70)	152 (68.4)	206(63.4)	148 (62.4)	1004 (68.2)
Positive	19 (20)	18 (34.6)	59 (25.2)	94 (30)	70 (31.6)	119 (36.6)	89 (37.6)	468 (31.8)
Total	95 (100)	52 (100)	228 (100)	313 (100)	222 (100)	325 (100)	237 (100)	1472 (100)

Table 2. Rotavirus strain distribution between the period January 2010 and December 2016.

VP7-type	VP4-Type					
	P[4]	P[6]	P[8]	P[Mix]	P[NT]	Total (%)
G1	3	45	104	1	5	158 (39.6)
G2	24	3	2	0	0	31 (7.8)
G3	0	19	1	0	0	20 (5)
G6	0	0	4	0	0	4 (1)
G8	0	0	1	0	0	1 (0.5)
G9	0	5	0	0	8	13 (3.2)
G10	4	0	0	0	3	7(1.7)
G12	1	43	60	1	3	108 (27)
GMix	0	7	15	7	3	32 (8)
GNT	2	4	14	5	0	25 (6.3)
Total (%)	34 (8.5)	126 (31.6)	201 (50.4)	15(3.7)	23 (5.8)	399 (100)

GMix/P[Mix]: Multiple genotypes detected for either G, P or both; GNT/P[NT]: either G, P or both were nontypable

RESULTS

A total of 1472 <5 year old children were recruited in this study and 1472 stool samples were collected. Of the recruited children, the male sex was predominant about 57.4 %(845/627) .The sex ratio was 1.36. ELISA positive stool specimens were 31.8% (468/1472) (Table 1). RT-PCR and genotyping were performed on 85.2% (399/468) of the positive samples. 93.7% (376/399) of the tested samples were positive to VP7 with 6.3% (25/399) non-typable strains (Table 2) and 94.2% (376/399) were positive to VP4 and 5.8% (23/399) non-typable strains (Table 2). Regarding the VP 7 genotype, the G1 was predominant with 39.6% (158/399) followed by G12 with 27% (108/399) and Mix G 8% (32/399) (Table 2). Concerning the VP4, the predominant genotype was P [8] which was found in 50.4% (108/399) followed by P [6] 31.6% (32/399) (Table 2). Throughout the study period, rotavirus genotype G12 was most prevalent in 2012, 2013 and 2014, except the year 2010 when G9 was the most predominant genotype (Figure 1a). Genotypes G1, G2 and G3 were detected throughout the study period at varying frequencies. On the other hand, there was no change in the predominant P-type as P [8] remained dominant over the study period (Figure 1b). The predominant genotype combinations were G1P [8] with 26.1% (104/399); G12P [8], 15% (60/399); G1P [6], 11.3% (45/399) and G12P [6], 10.8% (43/399). The genotypes G3P [6] and G9P [6] were found at lower rate with 4.7 (19/399) and 1.2% (5/399) (Table 2).

DISCUSSION

WHO recommends that all countries introduce rotavirus vaccines into their national expanded program on immunization. This study was conducted as part of the sentinel surveillance of rotavirus diarrhea prior to the introduction of the vaccine in Côte d'Ivoire. The <5 year old children are more vulnerable to severe gastroenteritis with more serious consequences probably due to the fact that after 5 years they develop immunity due to natural rotavirus infections (Steel et al., 2016).

The prevalence of rotavirus diarrhea in this age group

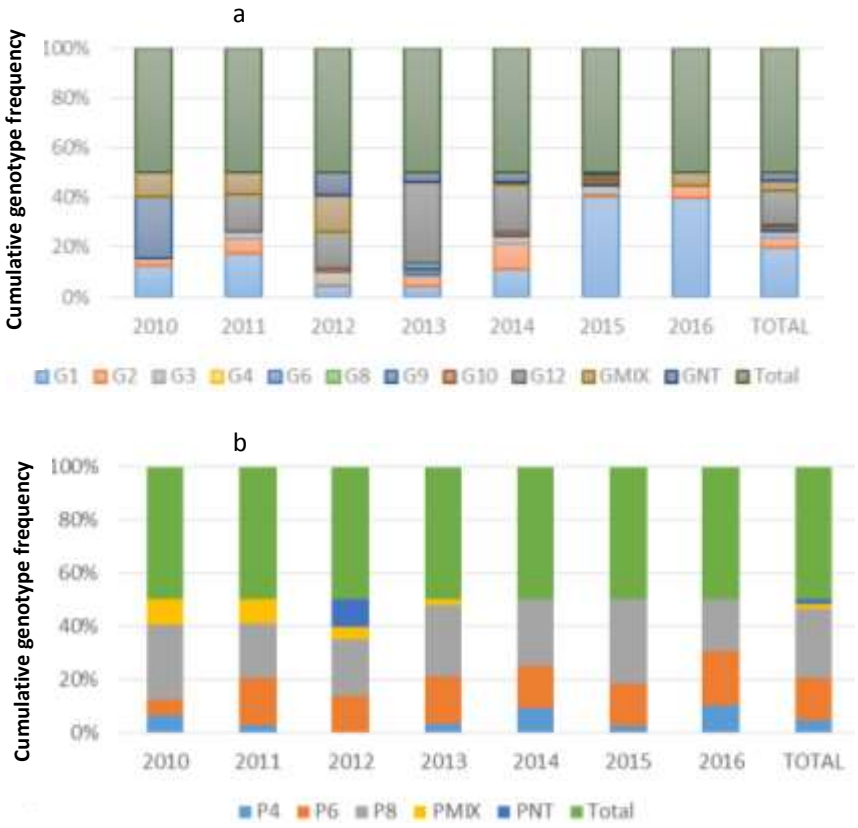

Figure 1. Temporal rotavirus genotype distribution in Côte d'Ivoire. a). Rotavirus G-type distribution, January 2010 to December 2016; b). Rotavirus P-type distribution, January 2010 to December 2013. MIX: multiple genotypes detected for either G, P or both; NT: either G, P or both were nontypable.

is high, particularly in the developing countries (Boula et al., 2014). Children under 12 months are the most affected by this virus (Todd et al., 2010). The peak of rotavirus gastroenteritis was found in some countries such as Kenya between 6 and 24 months (Agutu et al., 2017). In Cameroon, a prevalence of 44.7% was found in children under 24 months (Ndze et al., 2013). Hospitalization rates of 50.6% were observed in children aged 6-8 months in Ghana (Damaka et al., 2016). In this study, higher detection rates were observed than those found in previous studies in Côte d'Ivoire (Akoua-Koffi et al., 2007, 2014; Akran et al., 2010). The administration of the vaccine is therefore recommended in early infancy in sub-Saharan Africa as the infection is acquired early there than in Western countries (WHO, 2009). WHO recommends that the first dose of either RotaTeq or Rotarix be administered at age 6-15 weeks; the maximum age for administering the last dose of either vaccine should be 32 weeks (WHO, 2009). A predominance of male gender was observed in our study. This has also been observed in other studies (Selvarajan et al., 2017) but there was no statistical difference. Studies showed male susceptibility to rotavirus infection (Junaid et al., 2011). However, several studies have suggested the absence of gender-related occurrence (Saluja et al., 2014). Rotavirus is the primary cause of diarrhea in children under five years of age with a prevalence rate of 41% (Selvarajan et al., 2017) in area where vaccination is not yet introduced. The rate of 45% found in our study is similar to that found in other African countries before the introduction of the vaccine (Bwogi et al., 2016). However, in neighboring countries to Ivory Coast, where the vaccine has been introduced, rotavirus remains the leading viral cause of diarrhoea in children under five years of age, with high prevalence. In Burkina Faso, Ouedraogo found Rotavirus (63.5%), adenovirus (31.2%) and genogroup II norovirus (18.2%) in a study conducted (Ouedraogo et al., 2016). In Ghana, similar results have been found, but with lower prevalence rate of rotaviruses (27.9%), astroviruses (7.5%), noroviruses (6.8%) and adenoviruses (5.4%) (Akkufo et al., 2017). The outer layer protein, VP4 and VP7 of the group A rotavirus induce the production of neutralizing antibodies. The attachment protein VP 4 determines the type P. This has a more conservative specificity than type G determined by the glycoprotein VP 7. Ten G genotypes and 8 P genotypes have been detected in humans (Wylie et al., 2015). Two rotavirus vaccines are currently

licensed by WHO. RotaTeq® (RV5) (Merck & Co) consists of a mixture of 5 bovine viruses that contain VP7 and VP4 genes from human G1, G2, G3, and G4 and P viruses. Rotarix® (RV1) (GlaxoSmithKline Biologicals) consists of an attenuated virus derived from a human G1P [8] strain (Wylie et al., 2015). The two vaccines offer comparable protection against commonly circulating rotavirus serotypes G1- 4 [30]. The P [4], P [6] and P [8] genotypes are the most frequently found throughout the world (Abdel-Haq et al., 2003). The VP4 genotypes found in this study are similar to those circulating in the years 2000 to 2008 in Côte d'Ivoire. The P [4], P [6], and P [8] genotypes have been identified with similar rates in previous studies (Damanka et al., 2016; Kirkwood et al., 2014) with a predominance of P (Boula et al., 2014).

In the sub region there is a correlation of circulating VP4 genotypes with similar rates (Damanka et al., 2016; Enweronu-Laryea et al., 2013).Concerning the G-genotype, the G1 prevalence rate found in our study correlates with the results observed in other countries in the West African sub-region, particularly in Ghana (43%) (Laryea, 2013) and 46% in Cameroon (Enweronu-Laryea et al., 2013; Eteme et al., 2015). The G2 and G3 genotypes were found at rates >10% in the sub-region; in other African countries (Eteme et al., 2015; Damanka et al., 2016; Ngum Ndze et al., 2012) they were found at lower rates of 7.8% for G2 and 5% for G3, respectively. The G8 genotype present in the year 2000 in Côte d'Ivoire (Akoua-Koffi et al., 2007, 2014) virtually disappeared.

G12 genotype is a non-common strain with resurgence at 27%. This strain is not included in the target strains of the two vaccines recommended by WHO. Its emergence has been observed globally in several studies. In Thailand, Maneekarn et al. (2014) observed the predominance of the G12 from 2007 to 2009 only. In Australia, Kirkwood et al. (2014) reported incidence of G12P [8] (23%) in 2012, and Wylie (2014, 2015) reported an emergence of this strain in the same year in the Saint Louis, United States. This emergence was also observed in Africa, particularly in Cameroon in the same period (Ngum Ndze et al., 2012). Its significance is yet to be evaluated in the post vaccine era. Concerning genotypic combinations, the G1P [8], G12P [8], G1P [6] and G12P [6] genotypes are predominant in our study. The association of the genotype G12 with the different genotypes P suggests a high capacity of adaptation of this unusual strain. In this study, a lower rate was observed for non-typable and mixed strain.

Conclusion

Continuous monitoring of circulating strains is important as vaccine pressure may lead to the emergence of new epidemic strains in the post vaccine era. The determination of the different genotypes of rotavirus strains before the introduction of vaccination is fundamental to better understand the mechanisms leading to the emergence of new strains. Countries are encouraged to monitor strains in circulation before and after the introduction of the vaccine to determine the impact of the vaccine on circulating strains which might potentially escape protection covered by the currently recommended vaccines.

CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest.

ACKNOWLEDGMENTS

The authors acknowledge all the research assistants for recruiting patients in the Sentinel Surveillance Network Rotavirus Diarrhea in Cote d'Ivoire. The study received scientific support from the WHO Rotavirus Reference Laboratory of Ghana and South Africa. Financial support from WHO/AFRO for the supply of PCR reagents in reference laboratories is appreciated.

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