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The Antibiotic and Garlic Susceptibility of *Escherichia coli* Strains and Their Ability of Attachment and Biofilm Formation

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Abstract: This study consisted on the impact of antimicrobial agents on *Escherichia coli* (*E. coli*) isolates and their power to adhere to surface for biofilms formation. Sixty two *E. coli* isolated from patients urines were then used for standard antibiograms on Mueller Hinton (MH) agar associated with garlic juice antimicrobial activity. The attachment of three *E. coli* strains selected according to their susceptibility to antibiotics was experimentally performed on glass slides and the adhesion profiles were determined to assess their power of biofilm formation. As results, even though, the dominant types of resistance were respectively to amoxicillin, tetracycline, ampicillin and nalidixic acid in more than 80% of all the *E. coli* isolates, the highest sensitivity were observed with Gentamycin in 81% and imipenem in 97% of all the samples. In addition, the tests of adhesion revealed that, significantly ($p < 0.0001$), the three *E. coli* attachment to surface increased with their resistance to antibiotics. However, none of them resisted the garlic juice as well as isogenic planctonic-growing bacteria as biofilm-ones.
Keywords: Antibiotic and garlic Susceptibility, *Escherichia coli* strains, ability, attachment, biofilm.

INTRODUCTION

The increase in multi-resistance to antibiotics leads to a serious threat to public health. It generates costs of care, prolonged stay in hospital with a hight rate of contamination and dissemination of multi-resistant germs through biofilms, therapeutic failures and sometimes deaths [1-3].

Biofilms are microbial communities in which, immobilized cells are irreversibly adhered to a surface and embedded in a self-produced matrix of extracellular glycoprotein, proteins and DNA. It was reported that, bacterial biofilms are responsible of chronic infections because they show high tolerance to antibiotics and chemical disinfectants as well as resisting phagocytosis and other human immune system than the planctonik bacteria. In fact, the polymeric substances generated from the microbial cells within the biofilm give them a compact structure to resist environmental stress, antibiotics and disinfectants and to escape the immune defense of the host[1, 4]. The attachment of bacterial cells is the determining step in biofilm formation. It depends on their ability to be readily adhered to the surface substratum and proliferate as well in abiotic as in biotic conditions [5].

In this study, we focused our investigations on three types of *E. coli* strains. Commonly, *E. coli* were supposed to be a commensal bacterium of the normal microflora as well in humans as in animals. However, some strains have resulted in serious morbidity and mortality with clinical diseases like gastroenteritis, sepsis/meningitis, cystitis and pyelonephritis. Until fairly recently, a common perception underlined that, pathogenicity traits increased in *E. coli* and become more the exception than rules [6, 7]. According to their resistance to antibiotics, bacterial attachment to a surface was performed on glass slides in order to detect which kind of the three *E coli* strains is more apt to form biofilm. In addition, the effect of garlic was tested in a promising strategy to seek another substance, capable of destroying the biofilm and having no harmful effect on the human body.

MATERIALS AND METHODS

Antimicrobial susceptibility testing and *E. coli* strains sampling

Sixty-two *E. coli* isolated on Mac Conckey medium from patients urines in CHR-Lomé Commune were sampled after identification using the "Bacterial Identification System (BIS®)" kit made in Belgium and purchased from Cypress Diagnostics. The disc diffusion

method was used to determine the antibiotic and garlic susceptibility profiles of each isolate. For this purpose, the inoculum of each isolate was prepared by sub-culturing one colony in peptone solution and incubating for 18 hours at 37°C and the inocula were prepared by introducing two drops of each isolate in 20 ml of sterile physiologic water (1/1000 diluted). Furthermore, a well was dug in the Mueller Hinton Agar (MHA) medium contained in the Petri dishes by using a 6 mm of diameter sterile tube. Thereafter, the agar (MHA) medium was sown by inundation with 4 ml of each inoculum. The excess of inoculum was re-sucked and the dishes were air-dried at 37°C until 15 min.

On the other hand, the garlic juice was prepared as carried out by Olayemi and Opaleye (1999). A measure of 20g of the garlic (*Allium sativum*) bulb was introduced into a beaker, containing 80ml of distilled water. The mixture was soaked for 72hours after which the solution was carefully filtered with muslin cloth into a sterilized conical flask.

The garlic juice was then introduced into each well and the antibiotic discs were applied on inoculated medium by respecting at least a gap of 30 mm between either two discs or a disc and the well to avoid the inhibition zones overlapping. After 15 min of pre-diffusion at room temperature, the dishes were incubated at 37°C for 18 hours. Finally, the diameter of inhibition zones was measured and the results were interpreted according to the standard norms of the Clinical and Laboratory Standards Institute (CLSI) [8]. The antibiotics that were tested, included Amoxicillin (AMX 25), Amoxicillin + Clavulanic Acid (AMC 20/10), Ampicillin (AMP 10), Ceftazidime (CAZ 30), Ceftriaxone (CRO 30), Cefoxitin (FOX 30), Cephalothin (KF 30) Amikacin (AK 30), Gentamycin (CN 5), Cefotaxime (CTX 30), Nalidixic Acid (NA 30), Imipenem (IMP10), Tetracycline (TE 30) and Ciprofloxacin (CIP 5) and the control quality was performed by using the reference strain ATCC25922 of *E. coli*. The *E. coli* isolates were stored at -80°C in Tryptic Soy Broth (TSB; Biokar Diagnostics, Pantin, France) containing 40% (v/v) of glycerol for further uses.

Bacterial strains used for adhesion assays and *inoculum*s preparation

Bacterial adhesion method used was the one described by Hamadi *et al*. [5]. The three *E. coli* strains used for the adhesion assays were selected from the sixty-two sampled in step 2.1. according to their antibiotic susceptibility. The most sensitive strain was Ec55LC, inhibited by Tetracycline to which all other strains resisted; Ec27LC was the strain that was resistant to antibiotics inhibiting 50% of samples and Ec44LC was the most resistant strain that escaped Imipenem (Table I). The three strains were stored at -80°C in Tryptic Soy Broth (TSB; Biokar Diagnostics, Pantin, France) containing 40% (v/v) of glycerol. Pre-cultures were prepared by inoculating 100 µl from frozen stock cultures tubes into 5 ml of TSB and incubating at the same temperature of culture (37°C). The pre-cultures were incubated at 37°C for 24 h. The cultures used in each experiment were then prepared by inoculating $5x10^4$ CFU/ml from the pre-culture broths into 50 ml of TSB in sterile 500 ml flasks. Cultures were incubated under shaking (160 rpm) at 37 °C and stopped at the late exponential phase.

Bacterial standardization for slides inoculation

E.coli strains grown at 37°C as described previously were harvested by centrifugation for 10 min at 3500 *g* (20°C). Bacteria were washed twice with 20 ml of 100 mM potassium phosphate buffer (PB) (pH 7) and finally resuspended in 20 ml of PB. The cells are dispersed by sonication at 37 kHz for 5 min at 25°C. Subsequently, bacteria were resuspended in the PB to a cell concentration of $1x10^8$ CFU/ml by adjusting the optical density to OD620nm= 0.110 ± 0.005 (10^8 CFU/ml) using an UV/visible light spectrophotometer. Standardized cell suspensions were diluted 10 fold for use in the bacterial adhesion assays (10^7 CFU/ml).

Slides preparation and adhesion assays

The glass slides were cleaned by soaking in ethanol 95° overnight to remove grease. The slides were then thoroughly rinsed five times for 1 min with agitation in 500 ml of distilled water followed by three washes with ultrapure water (Milli-Q® Academic, Millipore, France). The clean slides were air-dried and sterilized by autoclaving at 121°C for 15 min. The sterile slides were placed in a horizontal position in Petri dishes. The upper face of each slide was covered with 3 ml of cell inoculums (10^7 CFU/ml) and incubated statically at 37°C for 60 min for the bacterial adhesion assays. After attachment, the coupons were removed using sterile forceps and rinsed by gently dipping into PB to remove excess liquid droplets and unattached cells. The adhered cells were then stained for 10 min in the dark using acridine orange stain 0.01% (w/v), followed by gently dipping in 30 ml of ultrapure water. The attached cells were quantified using epifluorescence microscopy. A total of 50 fields per coupon were scanned and the fluorescent cells were enumerated. Counts were presented as number of bacteria in microscopy field. The results present the average of three independent experiments and two coupons were studied for each experiment.

Biofilm assays and inhibitory activity of garlic on cells taken off

After the adhesion assays as described previously, attached cells on slides were covered with 2 ml of TSB and incubated statically at 37°C for 24 hours for the biofilms formation. Hence, slides were removed and rinsed by gently dipping into PB to remove excess liquid and unattached cells. Thereafter, slides were soaked again in PB with agitation to take off cells from biofilms. These cells were harvested through

centrifugation and resuspended in 20 ml of PB. After sonication at 37 kHz for 5 min at 25°C to disperse cells, 0.5 ml of a cell concentration adjusted to the optical density of OD620nm= 0.110 ± 0.005 (10^8 CFU/ml) using an UV/visible light spectrophotometer was added to 0.5 ml of the garlic extract (v/v) for an hour and the mixture optical density (mOD) was determined. Then, 4 ml of each mixture was reinoculated dishes containing MHA to detect the inhibitory activity of garlic. Dishes containing MHA were also reinoculated with 4 ml of each cell concentration without the garlic extract as control.

Statistical analyzes

Data analysis was performed using the software JMP (SAS Institute Inc.version 5.0.1a, Cary, NC, USA). The bacterial counts were transformed in natural logarithms and then, the means and standard deviations and also the median, lower and upper values of each cell adhesion were computed.

RESULTS

All the 62 *E. coli* strains enrolled in this study were found resistant to amoxicillin; only one strain (Ec55LC) presented a marked susceptibility to tetracycline in 15% of isolates to which the other strains resisted. A sensitivity of identical proportion (15%) was observed between amoxicillin associated with clavulanic acid and tetracycline but not strictly with the same isolates in our sampling. The third dominant types of resistance were to ampicillin and nalidixic acid also identically detected in 81% of isolates. The susceptibility of *E. coli* isolates to cephalothin as ceftriaxone (24% of samples) was respectively followed by these of cefotaxime (52%), ciprofloxacin (58%), ceftazidime (63%), amikacin (74%), cefoxitine (76%). The highest antibiotic inhibitions power were observed with Gentamycin in 81% and particularly with Imipenem in 97% of samples used in our study (Figure 1). In our sampling, out of 13 antibiotics commonly used (amoxicillin excluded) for enterobacteria antibiogram, 7 (54%) showed clear inhibition on half of *E. coli* strains.

In the other hands, among the strains chosen for adhesion assays, the strain Ec55LC susceptibility was followed by Ec27LC that was resistant to antibiotics inhibiting 50% of samples (cefotaxime). With regards to the strain Ec44LC, it was the most resistant that escaped the Imipemen antibiotic (Table I). However, more than the gentamicine and imipenem, which respectively inhibited 81 and 97% of the *E. coli* germs, the crude extract of garlic was active on hundred percent of our sampling.

For the adhesion assay, 50 fields per coupon scanned and the fluorescent cells enumerated on 50 slides, revealed the number of adhered bacteria to glass slides that were logged. The mean values and their standard deviations, median values, the lower and upper values were expressed on Figure 2. As results, the strain Ec55LC presented a mean value of 2.149 $\pm$ 0.037 against 2.941 $\pm$ 0.044 for the strain Ec27LC and 3.327 $\pm$ 0.043 for the strain Ec44LC at a significance level of 95%. Similarly, the median values, the lower and upper values increased significantly according to the strain resistance. The difference between these values was statistically significant ($p< 0,001$). In addition, when the garlic extract was added to the *E. coli* cells taken off from the biofilms and reinoculated TSA, no culture was observed during 24 hours comparatively to the respective control strains to which the garlic juice was not added.

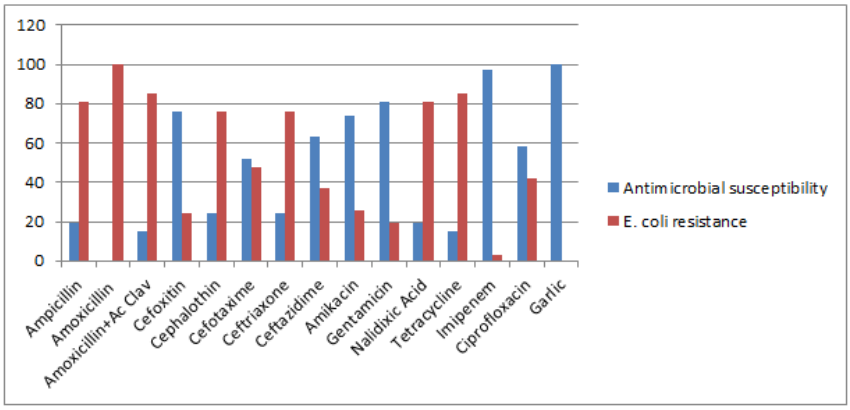

Fig-1: Antimicrobial susceptibility of *Escherichia coli* isolates

Table-I : Antimicrobial effect on *Escherichia coli* strains used for adhesion

	AMX 25	TE 30	CTX 5	CIP 5	CN 500	IMP 10	GARLIC
MIC	R< S ≥	R< S ≥	R< S ≥	R< S ≥	R< S ≥	R< S ≥	R< S ≥
DZI	16 - 19	17 - 19	17 - 20	22 - 25	14 - 17	16 - 22	16 - 22
Ec55LC	0	19	23	26	21	25	26
Ec27LC	0	0	22	26	20	24	26
Ec44LC	0	0	15	13	14	17	24

MIC = Minimal Inhibitory Concentration, DIZ = Diameter of Inhibition Zone, R = Resistance, S = Sensitivity, AMX= Amoxicillin, TE= Tetracycline, CTX= Cefotaxim, CIP= Ciprofloxacin, CN= Gentamycin, IMP= Imipenem.

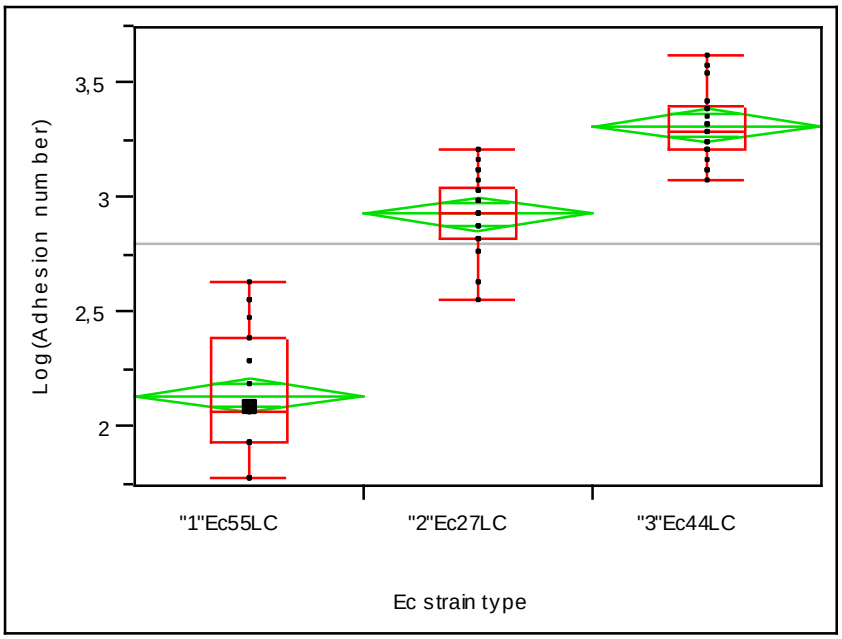

Fig-2: Adhesion of *Escherichia coli strains* according to their resistance to antibiotics

DISCUSSION

Antibiotic resistance is one of world health concern in particular in *E. coli* because this microbe is the most common enteropathogen in humans. It is also the most responsible cause of urinary tract infections, sepsis and a common cause of nosocomial and community-acquired bacteraemia as well as a pathogen that causes diarrhea especially in children [9, 10]. This study did not focus on the determination of multidrug resistance because its aspects have already been addressed by many other studies. It was conducted not only to determine the antibiotic sensitivity of *E. coli* isolates but also to test the effectiveness of garlic as a natural alternative to currently used antibacterial substance for planktonic *E. coli* strains and these bacteria in biofilm.

Through their susceptibility to antibiotics and garlic the attachment and biofilm formation ability of three *Escherichia coli* (*E. coli*) strains was assessed on glass slides. The susceptibility profile to antibiotics was sought through the culture of each *E coli* strain on MHA using inhibition zone measurement method. Increasing resistance to antimicrobial agents especially the first-line antibiotics among *E. coli* strains represents a potential threat to public health [1, 9, 10]. In our study, all the *E coli* isolates found were resistant to amoxicillin; only one makes the exception among sixty-two with a marked susceptibility to tetracycline. Several studies have largely emphasized the predominant microbial resistance to amoxicillin, ampicillin and tetracycline [11, 12]. However, the susceptibility to amoxicillin was increased (15% of all the samples against nothing) when it was used in association with clavulanic acid; hence, it is strongly recommended to use this combination when using amoxicillin to avoid the discrepancies between standard antibiograms and an accurate minimal inhibition concentration (MIC) [1].

Although the proportion of resistant *E. coli* is the same for cephalothin and ceftriaxone, it does not relate to the same isolates. In addition, cephalothin is from the first generation cephalosporins when ceftriaxone is from the third generation. Moreover, ceftriaxone as cefotaxime, ceftazidime, cefoxitin and imipenem are antibactericidal antibiotics and beta-lactamins of third generation-cephalosporins, but they acted very differently. In terms of proportion, when ceftriaxone acted less than 50% of the *E. coli* sampling, cefotaxime inhibited 50% and the others were active on

60% and more. This may depend on their mode of action as well as the uncontrolled use of one with respect to another [6].

Only one isolate escaped imipenem that was the most sensitive of our conventional antibiotic panel used for antibiogram testing. Imipenem remains very stable in the presence of β-lactamases produced by some bacteria (penicillinase and cephalosporinase), and is a strong inhibitor of β-lactamases from some Gram-negative bacteria that are resistant to most β-lactam antibiotics [13]. In recent years, many studies revealed that, the resistance to cephalosporins in enterobacteriaceae members has considerably increased particularly due to the spreading of Extended-spectrum β-Lactamases (ESBL) [4, 13]. According to their results, bacteria harboring Extended Spectrum β-Lactamase (ESBL) enzymes are multi-drug resistant and that could pose serious problems for treatment and of course a challenge to the public health [14]. Apart from cephalosporins, excepted nalidixic acid that inhibited less than 50% of samples, ciprofloxacin, amikacin and gentamicin acted effectively on more than 50%. Ciprofloxacin is an antibiotic of the quinolone family widely used; this could reduce its antimicrobial power on many pathogens. In our case, the highest proportion of the imipenem antimicrobial effectiveness was just followed by these of gentamicin. The bactericidal activity of gentamicin works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. This justifies its choice among the most effective and safe medicines needed in a health system [15]. In addition, there is a similarity of mechanism of action between gentamicine and other aminoglycosides as amikacin [16].

Despite the wide susceptibility power of some antibiotics used for our antibiogramms, no of them induces a clear sensitivity on all the 62 isolates in our study [17]. Hence, the need to investigate the antimicrobial effect of garlic on our *E. coli* samples becomes crucial.

It was well established that garlic has a wide spectrum of actions; not only is it antibacterial, antiviral, antifungal and antiprotozoal, but it also has beneficial effects on the cardiovascular and immune systems [18]. In our study, for all the 62 *E. coli* isolates, clear zones of inhibitions were found around the wells in which the garlic juice was introduced. This revealed that, both the 62 isolates were sensitive to the garlic juice. Our study was in accordance with Harris JC *et al*. [18] who, through their investigations proved that, *Allicin*, one of the main active principles of the garlic juice homogenates has an antimicrobial activity on multidrug-resistant strains especially enterotoxicogenic *Escherichia coli*. However, Akintobi *et al.* [19] did not found the effectiveness of the garlic activity on *E. coli* strains.

To improve the effectiveness of garlic antimicrobial activity as a natural alternative, we investigated on *E. coli* biofilms by using its crushed homogenate extract. Multidrug resistance in *E. coli* as reported worldwide by many findings suggests that biofilms are source of contamination and dissemination of resistant bacteria. In order to prevent chronic infections related to *E. coli* contaminants and to reduce the risk of antimicrobial resistance, more knowledge is needed on the influence of the first stage of biofilm formation (i.e. the adhesion power of *E. coli* stains according to their resistance to antimicrobial agents) [5]. Our investigations in accordance with several studies revealed that, the increase of the main values of microbial attachment on glace slides may lead to their resistance to antibiotics [20, 21]. In fact, the mutation frequency of biofilm-growing bacteria is significantly increased compared with planktonically growing isogenic bacteria and there is increased horizontal gene transmission in biofilms [4, 22]. These physiological conditions may explain why biofilm-growing bacteria easily become multidrug resistant by means of traditional resistance mechanisms against βlactam antibiotics, aminoglycosides and fluoroquinolones. Thus, bacterial cells in biofilms may simultaneously produce enzymes that degrade antibiotics, have antibiotic targets of low affinity and over express efflux pumps that have a broad range of substrates. Multi-drug resistance such as antibiotics, disinfectants and detergents may be acquired through mobile genetic elements such as plasmids, transposons, and class 1 integrons [23- 25].

However, the mixture (garlic juice and *E. coli* cells taken off from the biofilms) did not reveal any cell culture for the three strains comparatively to the respective controls to which the garlic juice was not added. This could mean the effectiveness of the garlic juice susceptibility even on resistant *E. coli* strains as well in planktinic medium as on their biofilm.

CONCLUSION

Antimicrobial resistant strains of *E. coli* are a serious matter of concern because resistance genes of a bacterial strain are easily transferable in biofilm to other strains. Pathogen cycling through food and medical devices is very common and might cause a potential health risk to people, where biofilms are the veritable sources. However, in our study, as resistant strains of *E. coli* in biofilm were revealed to be susceptible to garlic, further studies would be needed to confirm its effectiveness on these bacteria and to detect also its minimal inhibitory concentration of use.

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REFERENCES

1. Rasheed MU, Thajudin N, Ahamed P, Teklemariam Z, Jamil K. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. *Rev Inst Med Trop Sao Paulo*, 2014, 56(4): 341–346.
2. European Centre for Disease Prevention and Control (OCDC). Faits marquants sur la résistance aux antibiotiques. *Résumé des données récentes sur la résistance aux antibiotiques dans l'Union Européenne*, Stockholm. 2013.
3. Dourou D, Beauchamp CS, Yoon Y, Geornaras I, Belk KE, Smith GC. Attachment and biofilm formation by *Escherichia coli* O157 :H7 at different temperatures, on various food-contact surfaces encountered in beef processing. *International Journal of Food Microbiology* 2011, 149: 262–268.
4. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. *International Journal of Antimicrobial Agents*. 2010, 35: 322–332.
5. Hamadi F, Asserne F, Elabed S, Bensouda S, Mabrouki M, Latrache H. Adhesion of *Staphylococcus aureus* on stainless steel treated with three types of milk. *Food Control*. 2014, 38: 104-108.
6. Bonnet C, Diarrassouba F, Brousseau R, Masson L, Topp E, MS. Pathotype and Antibiotic Resistance Gene Distributions of *Escherichia coli* Isolates from Broiler Chickens Raised on Antimicrobial-Supplemented Diets *Applied and environmental microbiology*, 2009, 75: 6955–6962.
7. Hamelin K, Bruant G, El-Shaarawi A, Hill Stephen, Edge TA, Bekal Sadjia. A Virulence and Antimicrobial Resistance DNA Microarray Detects a High Frequency of Virulence Genes in *Escherichia coli* Isolates from Great Lakes Recreational Waters. *Appl Environ Microbiol*. 2006; 72: 4200-4206.
8. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Seventh Informational Supplement. 2007, CLSI document M100-S17.
9. Sayah, RS, Kaneene JB, Johnson Y, Miller R. Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic- and wild-animal fecal samples, human septage, and surface water. *Appl. Environ. Microbiol*. 2005, 71:1394–1404.
10. Österblad M, Hadanen A, Manninen R, Leistevuo T, Peltonen R, Meurman O. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *J Antimicrob Chemother*. 2000, 44:1479–1484.
11. Ferjani A, Marzouk M, Moussa FB, Boukadida J. Antibiotiques resistance of *Escherichia coli* isolated from the urinary tract to amoxicillin–clavulanic acid and other antibiotics. *Médecine et Maladies Infectieuses*, 2010, 40 (3): 161-164
12. Karami N, Nowrouzian F, Adlerberth I, Wold AE. Tetracycline Resistance in *Escherichia coli* and Persistence in the Infantile Colonic Microbiota. *Antimicrobial agents and chemotherapy*. 2006, 50 (1) : 156–161.
13. *Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME. "Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to Enterobacteriaceae producing extended-spectrum β-lactamases: a systematic review and meta-analysis".* The Journal of antimicrobial chemotherapy. *2012, 67 (12): 2793–803.*
14. Yusha'u M, Umar MI, Suleiman K. Indigenous commercial drinks as potential sources of extended spectrum β-lactamases (ESBLS) producing organisms in Kano, Nigeria. *Int J Biomed Health Sci.* 2010;6:103–108.
15. "WHO Model List of Essential Medicines (19th List)". World Health Organization. *April 2015.* Archived *from the original on 13 December 2016*, Retrieved 8 December 2016.
16. "Gentamicin sulfate" The American Society of Health-System Pharmacists. Archived *from the original on 2015-08-16*. Retrieved Aug 15, 2015.
17. Oteo J, Delgado-Iribarren A, Vega D, Bautista V, Rodríguez MC, Velasco M. Emergence of imipenem resistance in clinical Escherichia coli during therapy. *Int J Antimicrob Agents*. 2008, 32(6):534-537. doi:10.1016/j.ijantimicag.2008.06.012.
18. Harris JC, Cottrell SL, Plummer S, Lloyd D. Antimicrobial properties of Allium sativum (garlic). Appl Microbiol Biotechnol. 2001, 57(3):282-286.
19. Akintobi OA, Nwanze JC, Ogele JO, Idowu AA, Onianwa O, Okonko IO. Antimicrobial Activity of *Allium sativum* (Garlic) Extract against Some Selected Pathogenic Bacteria. *Nature and Science*. 2013, 11(1) : 7p.
20. Liu P, Soupir ML, Zwonitzer M. Association of Antibiotic Resistance in Agricultural Isolates with Attachment to Quartz. *Appl Environ Microbiol.* 2011, 77: 6945-6953.
21. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. *Lancet* 2001, 358: 135–138.
22. Madsen JS, Burmolle M, Sorensen SJ. A Spatiotemporal View of Plasmid Loss in Biofilms and Planktonic Cultures. *Biotechnology and Bioengineering.* 2013, 110 : 3071-3074.
23. Lewis K. Persister cells, dormancy and infectious disease. *Nature Reviews Microbiology.* 2007, 5: 48-56.
24. Elexson N, Afsah-Hejri L, Rukayadi Y. Effect of detergents as antimicrobial agents on biofilm of antibiotics-resistant *Vibrio parahaemolyticus* isolates. *Food Control* . 2014, 35: 378-385.
25. Nadjir LK, Ayimba E, Alfa T, Dakey KA, Abozid MM, Pekpe KM. *Salmonella typhimurium* and *Staphylococcus epidermidis* biofilms resistance to Chlorinated water on plastic surface. *International*

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