

# Presence of coliform producing extended spectrum beta lactamase in sachet-water manufactured and sold in Abakaliki, Ebonyi state, Nigeria.

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Five different brands of sachet- water comprising of ten pieces per batch of each brand manufactured, sold and consumed in Abakaliki, Ebonyi State including My Summit, Gospel, Nnenna, Bewell and Age-less (NAFDAC Registered) were collected from each company in Abakaliki town and examined for the presence of Faecal coliforms using standard Microbiology technique. Isolated coliforms were later screened for production of extended spectrum beta lactamase enzymes using double disk synergy test method. Antimicrobial susceptibility patterns of both ESBL producing and non-ESBL producing strains were evaluated using disk diffusion method. A total of 11 (4.4%) strains of *Escherichia coli* were isolated from all the sachets examined, 3(1.2%) strains of *Enterobacter* spp were isolated from MS, GP and AG while 4(1.6%) of *Klebsiella* spp were isolated from MS, GP, BW and AG. 2(18 %) of *E. coli* isolated from Age-less sachet- water produced ESBL enzyme while no ESBL production was detected from *Enterobacter* and *Klebsiella* spp. Antimicrobial susceptibility studies showed that both ESBL producing and non-ESBL producing strains were resistant to all antibiotic tested showing a multi-drug resistance of both strains. Our findings are of serious public health concern which suggest that sachet-water should be well treated before selling to the public for consumption and also that more work should be carried out to determine the presence of resistant micro-organisms present in water.

**Keywords:** Sachet- water, faecal coliform, ESBLs, Susceptibility, Nigeria.

## INTRODUCTION

Extended spectrum beta lactamase enzymes were first reported in Germany in 1983 from *Klebsiella pneumoniae* and they are group of enzymes capable of hydrolyzing the third generation oxymino-cephalosporins such as (cefotaxime, ceftazidime, ceftriaxone), the monobactam (aztreonam) but not the cephamycin (cefoxitin, cefotetan) or carbapenems (imipenem, meropenem) (Kliebe et al., 1995; Bush, 2001; Bradford, 2001; Chaudhery and Aggarwae, 2004). These enzymes which are mainly produced by *E.coli* and *Klebsiella*

*pneumoniae* render this antibiotics in-effective when used to treat infection caused by ESBL producing organisms thus making therapy very difficult for clinicians. (Kim, et al., 2002; Liu et al., 2004). ESBLs have serine at their active sites which attack the amide bond in the lactam ring of antibiotics causing their hydrolysis. There are various reports of ESBL producing organisms worldwide from hospitals environments, patients, farm animals, sewages, food material (Chau and Oboegbunam, 2007, Mesa, et al., 2006, Hasman, et al., 2005).

Water is an important element of the physical environment which makes up a large proportion of our body total fluid (Chery and Le Chevallier, 2000). This substance is necessary in every aspects of mans activity;

therefore it needs to be free from pathogens, dissolved toxins, odour, colour and taste. Bottled or sachet- water is classified as food and is regulated and screened by National Agency for Food and Drug Administration and control in Nigeria, whose bacteriological standards are as recommended by World Health Organization (WHO, 1996). There are so many reports of sachet water and bottled water contamination by coliform bacteria that are resistance to various classes of antibiotics worldwide (Ash, 2002, Nwachukwu and Emeruem, 2007, El-Zanfaly, et al., 1998) but there is no report of organisms producing ESBL from water so far in this region. Therefore this study aimed at screening the presence of ESBL enzymes producing bacteria from sachet- water and their antimicrobial resistance patterns to different class of antibiotics.

## MATERIALS AND METHODS

### Collection of sachet- water samples

A total of 250 samples of sachet- water were collected from five different sachet- water producing companies in Abakaliki, Ebonyi State, Nigeria. 10 sachets each from 5 different batches of sachet- water were collected from these companies for a period of six months.

### Microbial analysis

An MPN presumptive test in MacConkey broth, MPN confirmatory test in brilliant green bile lactose broth and complete test using EMB agar followed by biochemical tests were performed for isolation and confirmation of coliform bacteria (Chessbrough, 2002).

### Phenotypic determination of ESBL enzymes

ESBL detection was determined on coliforms isolated from sachet- water namely *E. coli*, *Enterobacter* spps, *Klebsiella* spps using double disc synergy test. Briefly, a sterile Mueller-Hinton agar was prepared and a 0.5 MacFarland equivalent standard of the test organisms was streaked on the surface of the agar with a sterile loop and allowed for 15-20 mins to pre-diffuse. An Augmentin which is a combination of clavulanic acid 20 (µg) and amoxicillin (10 µg) was placed at the center of the petri-dish and cefotaxime (30 µg), ceftaxidime (30

µg), aztreonam (30 µg) ciprofloxacin (30 µg) were placed 15mm apart center to centre on the plates with a sterile forceps. These were incubated at 35°C for 18-24 h. An enhanced zone of inhibition from 5 mm above in the presence of Augmentin is regarded as positive for phenotypic production of ESBL enzyme.

### Antibiotic susceptibility studies

Sensitivity of *E. coli*, *Enterobacter* spp, and *Klebsiella* spp to different classes of antibiotics was performed by disc diffusion method. Briefly, a sterile Mueller-Hinton agar was prepared and a 0.5 MacFarland equivalent standard of the test organisms was streaked on the surface of the agar and allowed for 15-20 mins to pre-diffuse. The following antibiotics disc, gentamicin (10µg), erythromycin (5µg), trimethoprim (1.25µg), Sulphamethoxazole/trimethoprim (25µg), tetracycline (10µg), penicillin (10µg), clindamycin (20µg), ciprofloxacin (5µg), ceftazidime (30µg), cefotaxime (30µg) and aztreonam (30µg) were placed on the surface of the agar with a sterile forceps. These were incubated at 35°C for 18-24 h, after which the inhibition zone diameter in (mm) was taken.

## RESULTS

Out of 250 samples of sachet- water examined for the presence of coliform bacteria and those producing ESBL enzymes from five different brands of ten sachets per batch, 3(6%) of *E. coli* was isolated from batch 1 and 3 in My Summit, 3(6%) from batch 2 in Gospel, 2(4%) from batch 1 in Nnenne, 2(4%) from batch 3 in Age-less, 1 (2%) of *Klebsiella* spp was isolated from all the sachet water from batches 1-5 while 1(2%) of *Enterobacter* spp was isolated from all batches in My Summit, Gospel, and Age-less (Table 2). This finding shows that there is difference in sanitary quality of these sachet- water brands, from batch to batch as our result showed differences in numbers and types of organisms present in different batches. ESBL determination studies showed that 2(18%) of *E. coli* isolated out of 11 isolated were ESBL producers, these strains were isolated from Ageless sachet- water from two different batches (1 and 3) (Table 3). This is one significant finding from this study that is of serious public health concern. Also antibiotic susceptibility studies showed that all the coliforms isolated were totally (100%) resistant to all antibiotics tested showing multi-drug resistance from both ESBL and non-ESBL producing strains (Table 4).

**Table 1.** Group classification of bacteria isolated from each sachet water batch

| Names and number of organism isolated | Batch group | Sachet water in each batch group |
|---------------------------------------|-------------|----------------------------------|
| <i>E. coli</i> nos 1-11               | 1-3         | MS,AG, GP,NN, BW                 |
| <i>Enterobacter</i> spp               | 4           | Ag, MS, GP                       |
| <i>Klebsiella</i> spp                 | 5           | AG, MS, GP, BW                   |

KEY:MS-Mysummit,AG-Age-less,GP-Gospel,NN-Nnenne,BW-Be-well

**Table 2.** Type, number and percentage of coliform bacteria growth on each sachet water sample

| Bacteria names          | Names of sachet water samples |        |        |         |         |          |
|-------------------------|-------------------------------|--------|--------|---------|---------|----------|
|                         | My summit                     | Gospel | Nnenne | Be-well | Ageless | Total    |
| <i>E.coli</i>           | 3(6%)                         | 3(6%)  | 1(2%)  | 1(2%)   | 3(6%)   | 11(4.4%) |
| <i>Klebsiella</i> spp   | 1(2%)                         | 1(2%)  | 0(0%)  | 1(2%)   | 1(2%)   | 4(1.6%)  |
| <i>Enterobacter</i> spp | 1(2%)                         | 1(2%)  | 0(0%)  | 0(0%)   | 1(2%)   | 3(1.2%)  |

**Table 3.** Percentage of ESBL positive isolates from each brand of sachet water.

| Bacteria names/nos        | Positive ESBL | Negative ESBL | Sachet water brands |
|---------------------------|---------------|---------------|---------------------|
| <i>E.coli</i> nos 1-3     | 0(%)          | 3(100%)       | My summit           |
| <i>E.coli</i> nos 4-6     | 0(0%)         | 3(100%)       | Gospel              |
| <i>E.coli</i> nos 7       | 0(0%)         | 1(100%)       | Nnenne              |
| <i>E.coli</i> nos 8-10    | 2(18%)        | 1(9.0%)       | Ageless             |
| <i>E.coli</i> nos 11      | 0(0%)         | 1(100%)       | Be-well             |
| <i>Klebsiella</i> spp 1   | 0(0%)         | 1(100%)       | My summit           |
| <i>Klebsiella</i> spp 2   | 0(0%)         | 1(100%)       | Gospel              |
| <i>Klebsiella</i> spp 3   | 0(0%)         | 1(100%)       | Be-well             |
| <i>Klebsiella</i> spp 4   | 0(0%)         | 1(100%)       | Ageless             |
| <i>Enterobacter</i> spp 1 | 0(0%)         | 1(100%)       | My summit           |
| <i>Enterobacter</i> spp 2 | 0(0%)         | 1(100%)       | Gospel              |
| <i>Enterobacter</i> spp 3 | 0(0%)         | 1(100%)       | Ageless             |

**Table 4.** Antibiogram of bacteria isolated from each sachet water brand

| Isolate groups                  | Antibiotics |     |      |     |     |     |     |     |     |     |     |
|---------------------------------|-------------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|
|                                 | GEN         | ERY | CLIN | TRI | SMX | PEN | CTX | CAZ | ATM | TET | CIP |
| Batch 1 <i>E.coli</i>           | R           | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |
| Batch 2 <i>E. coli</i>          | R           | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |
| Batch 3 <i>E.coli</i>           | R           | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |
| Batch 4 <i>Klebsiella</i> spp   | R           | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |
| Batch 5 <i>Enterobacter</i> spp | R           | R   | R    | R   | R   | R   | R   | R   | R   | R   | R   |

Key: GEN- gentamicin, ERY- erythromycin, CLIN: clindamycin, TRI: trimethoprim, SMX: sulphamethoxazole/trimethoprim, PEN-penicillin, CTX- cefotaxime, CAZ-Cefazidime, TET-tetracycline, CIP-ciprofloxacin, R- Resistant.

## DISCUSSION

Sanitary procedures and practices in the production line of all consumable products including water for drinking

should be the primary objective of all involved in such project as any defect could introduce micro-organisms which will be of serious health hazard to consumers. World Health Organization standard stated that all water

for human consumption should be totally free of coliforms but our study shows that this water produced and distributed do not met the required standard and as such not fit for human consumption. An outbreak of disease from drinking water has been reported worldwide, it was estimated that water-borne disease might account for one-third of the intestinal infections globally (Hunter, 1997). Another study reported that poor sanitation and hygiene were responsible for 40% of all deaths and 5.7 % of the total disease burden occurring worldwide (Pruss, 2002). The present study revealed that poor sanitary standard of sachet- water manufactured and distributed in Abakaliki by these companies, the isolation of coliforms indicates inadequate treatment of water or post treatment contamination and also could be contaminated from normal flora of personnel involve in the production because some of this coliform are present as normal flora of the body. Three different enteric organisms were isolated in low numbers from all the sachet- water although but this is very serious because of their high resistance to all the antibiotics tested which are common antibiotics used in treating infections caused by such organisms. This will pose a serious therapeutic problem when such antibiotics used for treating infections caused by these organisms. This study supported the results from other researchers in case of multi-drug resistance from water as reported in Aba and Owerri in South-eastern Nigeria (Nwachukwu and Emeruem, 2007). Antibiotic resistance has been reported in different parts of the world where *E. coli* was found to be resistant to all fluoroquinolones, aminoglycoside and some beta lactam antibiotics (El-Zanfaly et al., 1998)). The emergence of multi-drug resistance ESBL producing organisms in sachet- water produced in Abakaliki is a matter of grave concern. In Abakaliki the availability of water fit for drinking represent a problem for over three decades which had led to various outbreak of waterborne diseases, the worst was the endemic outbreak of guinea worms from early 80's to late 90's (unpublished data). Although low number of *E. coli* 2(18%) expressed ESBL enzyme, this signal is an urgent need for proper sanitary method of production and inspection of all water producing companies by NAFDAC to enforce that manufacturers produce water that meet WHO standards. This will help in eradicating the existence of these resistance organisms that could lead to a very serious public health problem in the near future. ESBL producing organisms are known world wide to harbor multi-drug resistance genes in plasmids, which confer resistance to wide range of antibiotics (Rooney et al., 2009). Non-ESBL producing organisms are known to be more susceptible to antimicrobial agents than ESBL producers because they lack the mutation that has occurred in the active serine site of ESBL producing organisms but in our results organisms found to be non-ESBL are also as resistant as ESBL producers. This suggest that these

organisms might be harbouring other ESBL genes which our method could not detect such as the carbapenamases, OXA, CMY etc. which are ESBL but DDST method is not suitable for their detection or some other genes that have developed resistance to the tested antibiotics. This suggests for more research work in this area using molecular technique to specifically find out the genes responsible for this grave resistance. Antibiotic resistance could be transferred from non-pathogenic bacteria to pathogenic ones by transfer of resistance genes in closely related species such as members of the *Enterobacteriaceae* family. To the best of our knowledge studies on ESBL producing organisms from water is scarce and this is so far the first study that evaluated the presence of ESBL producing organism from water in Nigeria and our findings call for urgent surveillance of presence of resistance organisms in water and for effective monitoring by regulatory bodies to make sure that water manufactured and distributed for drinking in Abakaliki conforms with WHO standard.

In conclusion, we detect for the first time ESBL producing organism from sachet- water manufactured and sold in Abakaliki and also non -ESBL producing organisms that are highly resistant to various classes of antibiotics.

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