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Full Length Research Paper

Presence of antibiotic resistant coliforms in sachet water sold in some parts of South Eastern Nigeria

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Three hundred sachet water samples produced in Enugu and Onitsha namely: Aqua Rapha, Bejoy, Teck Tack, SO and Rock Tama were collected from six different batches. They were screened for the presence of faecal coliforms using standard microbiology techniques. Sachet- water samples contaminated with faecal coliforms were selected and tested for their susceptibility to fourteen different antibiotics by disc diffusion method. 80 (26.6%) were contaminated with faecal coliforms as follows: Aqua Rapha 25 (41.6%) specifically (ESCHERICHA COLI, ENTEROBACTER and KLEBSIELLA spp.), Rock Tama 1 (1.6%) (E. COLI,), Bejoy 20 (33.3%) (E. COLI, ENTEROBACTER and KLEBSIELLA spp.) , Teck Tack 9 (15%) (E. COLI, ENTEROBACTER and KLEBSIELLA spp.), and SO 33 (55%) (E. COLI, ENTEROBACTER and KLEBSIELLA spp). Susceptibility studies showed that E. COLI was highly resistance to trimethroprim, sulphamethoxazole/trimethroprim, ampicillin, aztrenoman, ceftazidime, and cefpirome but susceptible to gentamicin, ciprofloxacin, cefoxitin and cefotaxime. ENTEROBACTER and KLEBSIELLA spp. were totally resistance to all the antibiotics tested. We report the presence of multi-drug resistance fecal coliform in the form of E. COLI, ENTEROBACTER and KLEBSIELLA spp. from sachet- water.

Key words: Sachet water, feacal coliform, antibiotics, multi-drug resistance.

INTRODUCTION

Sachet-water is available in sealed plastic material meant for sale for human consumption (Food and Drug Administration, 1995). Non-contamination with fecal matter is the most important parameter of water quality because human faecal matter is generally considered to be a great risk of human enteric pathogens (Scott et al., 2003). Water has played a significant role in the transmission of human diseases. Potential health problems may exist due to the microbial content of sachet water since water is one of the vehicles for the transmission of pathogenic organisms (Brock, 1991; Prescott et al., 2005). However, the type of organisms present depends on a number of factors such as the type of soil over which the water flows, contamination by animals, sewage and agricultural waste (Hunter, 1993). Water-borne diseases are associated with improper provision of water and sanitary services and the effect of these diseases vary in severity from stomach upsets to even death (Sonu et al., 2007). Most of the victims are young children especially from the developing world, an estimated number of more than 34 millions people die as a result of these water related diseases making it the leading cause of diseases and death around the world (WHO, 2005).

Efforts are being taken by all technological advancements including antibiotic usage to control transmission of water-borne diseases, but multi-drug resistance by some organisms such as *Escherichia coli*, warrants the

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beginning of steps to prevent public health hazards (Tambekar et al., 2006; Pandey and Musrat, 1993). The use, misuse and under-use of antibiotics are responsible for resistance development to bacterial antimicrobials worldwide. Antibiotic resistance has been reported in Acinetobacter, Alkaligenes, Citrobacter, Enterobacter, Pseudomonas, Serratia spp. isolated from river (Paven et al., 1997). Antibiotics used in poultry or agriculture practice to prevent disease can contaminate surface and underground water (Ash, 2002) from where they can be transported to humans in drinking water thereby adding to resistance problems. In the present study we aimed to evaluate the bacteriological quality of sachet water by isolating fecal coliforms (as indicator of fecal contamination) in sachet- water manufactured and distributed in some parts of South Eastern Nigeria (Enugu and Anambra) and there possible association with drug resistance.

MATERIALS AND METHODS

Collection of sachet water

Three hundred samples of sachet water were collected from different companies (Bejoy, Aqua Rapha, Teck Tack, SO and Rock Tama) from South Eastern Nigeria (Enugu and Anambra).

Microbial analysis

Sixty water samples were each collected from five different sachet water brands. Ten samples each were collected from six different batches and was analyzed for the presence faecal coliform using standard Microbiology techniques namely: MPN presumptive test in MacConkey broth, MPN confirmatory test in brilliant green bile lactose broth and MPN complete test using EMB agar. Further characterization was carried out by biochemical test reactions namely indole test, methyl-red test, Voges-Proskauer test, citrate utilization test, motility and Gram staining (Cheesbrough, 2002).

Antibiotic susceptibility studies

Sensitivity of faecal coliforms isolated from sachet- water namely *E. coli*, *Enterobacter* and *klebsiella* spp. to different classes of antibiotics was performed by disc diffusion method. Briefly, 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 min to pre-diffuse. The following antibiotics disc, gentamicin (10 µg), trimethoprim (5 µg), Sulphamethoxazole/trimethoprim (25 µg), ampicillin (10 µg), cefpirome (30 µg) ciprofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg) cefoxitin (30 µg)and aztreonam (30 µg) were placed on the surface of agar plates with a sterile forceps. These were incubated at 35°C for 18 - 24 h, after which the inhibition zone diameter in (mm) was taken and interpreted using CLSI standard (Bauer et al., 1966).

RESULTS

Our result revealed a high level of faecal coliform contamination in SO 33 (55%), Aqua Rapha 25 (41.6%), and Bejoy 20 (33.3%) (Table 1). This showed very poor sanitary standard of operation in the production line of sachet- water, also the presence of the same type of fecal bacteria in all batches shows common source of contamination. Low level of contamination was observed in Rock Tama 1 (1.6%) and Teck-Tack 9 (15%), this could be attributed to some level high sanitary standard and proper treatment employed in the production line of this sachet- water (Table 1). Susceptibility studies showed that *E. coli* were susceptible to gentamicin (80%), ciprofloxacin (86%), cefotaxime (87%) and cefoxitin (95%), but resistant to trimethoprim (91%),

Sulphamethoxazole/trimetoprim (82%), tetracycline (100%), ampicillin (94%), aztreonam (85%), ceftazidime (90%), cefpirome (90%). *Enterobacter* and *Klebsiella* spp. were completely resistant to the entire antibiotic tested. This reveals the presence of multi-drug resistant bacteria that are capable of inactivating various kinds of antibiotics (Table 2).

DISCUSSION

This study suggests that most of the sachet-water produced in some parts of South-Eastern Nigeria (Enugu and Anambra) are of very poor sanitary standard and as a result not fit for human consumption. These could serve as an important source of water-borne diseases like diarrhea, gastroenteritis and dysentery. Drinking-water from some parts of South Eastern Nigeria are of poor sanitary quality as observed from our study as such confirms the finding of some researchers in other parts of South- Eastern Nigeria (Aba and Owerri) (Nwachukwu and Emeruen, 2007). Our findings shows that it is necessary to determine the presence of faecal coliform from water intended for drinking in order to prevent faecal coliforms on regular basis. From our findings, it could be deduced that Rock Tama is of good quality for human consumption despite finding a few coliforms in one of the batches; this shows some degree of high standard of sanitary procedure in production as other batches were devoid of fecal coliforms. This should not be ignored to avoid subsequent contamination of some other batches in the near future. Some level of good sachet-water quality was also observed in Teck Tack, 9 sachets were contaminated from one particular batch with the same type of faecal coliform showing contamination from a common source. Sachet water from Bejoy, Aqua Rapha and SO were of very poor sanitary standard as the entire batches were contaminated with faecal coliform, this shows that they are not fit for human consumption. The emergency of multi-drug resistance among these bacteria spp. and their presence in drinking water is of serious health-care concern. Multi-drug resistant *E. coli* producing extended spectrum beta lactamase from sachet water was recently reported in Abakaliki, Ebonyi State, Nigeria

Table 1. Percentage of sachet water brands contaminated with feacal coliform.

Sachet water brands	Percentage of positive sample (%)	Percentage of negative sample (%)	Fecacal organism isolated
Aqua Rapha	25 (41.6)	35 (58.4)	*E. coli*, *Enterobacter* spp. *Klebsiella* spp.
Bejoy	20 (33.3)	40 (66.7)	*E. coli*, *Enterobacter* spp. *Klebsiella* spp.
Teck Tack	9 (15)	51 (49)	*E. coli*, *Klebsiella* spp.
SO	33 (55)	27 (73)	*E. coli*, *Enterobacter* spp. *Klebsiella* spp.
Rock Tama	1 (1.6)	1 (99)	*E. coli*,

Table 2. Percentage of bacteria resistant to different antibiotics.

Names of antibiotic discs (µg/ml	Percentage resistance of coliform bacteria		
	E. coli (%)	*Enterobacter* spp. (%)	*Klebsiella* spp. (%)
Gentamicin (10)	20	100	100
Trimethoprim (5)	91	100	100
Sulphamethoxazole/Trimethoprim (25)	82	100	100
Ciprofloxacin (5)	14	100	100
Aztreonam (30)	85	100	100
Ceftazidime (30)	90	100	100
Cefpirome (30)	90	100	100
Cefotaxime (30)	13	100	100
Cefoxitin (30)	5	100	100
Ampicillin (10)	94	100	100

(article still under-review). These may be the reason for some out-break of water borne disease in this region (un-published data). ESBLs may confer resistant genes to other fecal coliforms other than ESBL because of the fact that they carry genes responsible for resistance to various classes of antibiotics and may be responsible for the resistance observed. The prevalence of drug resistant organisms has imposed a great challenge to clinicians; this study provides a guide to the possible cause of antibiotic resistance by bacteria which could be prompted by drinking poorly treated or untreated packaged water which could prolong the treatment of water-borne diseases. The presence of organisms in drinking water is of public health importance, as it is mostly responsible for *Staphylococcal* food poisoning (El-Zarfaly et al., 1998; Hobb and Robert, 1993; Frazier and Westhoff, 1995). WHO (1996) guideline value for bacteriological quality for all water intended for drinking states that *E. coli* or total coliform bacteria must not be detected in any 100 ml samples. Therefore, by this standard, 80% of the sachet-water tested is not safe for human consumption. It is not surprising in our study that bacteria isolates from water were resistant to commonly used antibiotics as such has been previously reported in some other parts of South-Eastern Nigeria (Nwachukwu and Emeruen, 2007). This implies that treatment of water-borne diseases with these antibiotics may be inappropriate and will require new antibiotics which are not commonly used. Antibiotic resistant bacteria are a cause for concern because of possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more multiple antibiotic resistant organisms (Mckeon et al., 1995)

We conclude that majority of sachet-water distributed in some parts of South Eastern Nigeria, are not of good sanitary quality for consumption as they harbour faecal coliforms with multiple resistance to different classes of antibiotics. Therefore we suggest that more effort is needed to limit the number of contaminated sachet-water in distribution by ensuring that manufacturers of such products are properly supervised by regulatory agencies to ensure that they apply the standard methods established by WHO so as to meet the standard zero coliform presence in water.

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