**Sachet Water Syndrome: A Potential Vehicle for the Transmission of Antibiotic Resistance Pathogenic Organisms**

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**Abstract:** A total number of 54 sachet water samples from nine brands randomly purchased from different sales points in Mubi metropolis were used for this study. A volume of each sample was added to an equal volume of bacteriologic peptone water and incubated at 37oC for 24hrs and streaked unto McConkey agar and Mannitol salt agar for differential purposes. All the sachet water samples had growth on McConkey agar, while 30(55.5%) of the samples had no growth on Mannitol salt agar. Seventy eight (78) isolates in all were isolated from the 54 sachet water samples which include *Staphylococcus* *aureus*, Coagulase-negative Staphylococci (CoNS), *Pseudomonas* aeruginosa, *Escherichia* *coli*, *Proteus* *vulgaris*, *Shigella* spp., *Salmonella* spp. and *Citrobacter* spp. Among the isolates, 24(30.8%) are gram positive, while 54(69.2%) are gram negative. Coagulase negative Staphylococci (CoNS) have the highest incidence rate (70.8%) among the gram positive isolates, while *Pseudomonas* *aeruginosa* has the highest incidence rate (24.1%) among the gram negative isolates. On the over all, CoNS has the highest incidence rate (21.8%), while *Escherichia* *coli* and *Salmonella* spp. has the least incidence rate (6.4%). The results of the antibiotic susceptibility testing revealed that both *Staphylococcus* *aureus* and CoNS were 100% resistant to Penicillin-G, Amoxicillin, Amoxicillin-Clavulanic acid, Cotrimoxazole, Cephalexin, Cefazolin and Cefuroxime; their resistance to these antibiotics were however not significantly different from that of Erythromycin (p>0.05), but were significantly higher than those of other antibiotics (p<0.05). Also resistance by CoNS and *Staphylococcus* *aureus* to Chloramphenicol, Tetracycline and Azithromycin were not significantly different (p>0.05), but were significantly higher than those of Ciprofloxacin, Ofloxacin, and Piperacillin (p<0.05), and significantly lower than those of other antibiotics (p<0.05). Also, Seventeen (17) resistance patterns were shown by both *Staphylococcus* *aureus* and CoNS with ck,pg,ax,ac,ct,cp,cf,cr,er,te,az as the most common resistance pattern. The antibiotic susceptibility testing of gram negative isolates showed that all of them are 100% resistance to Nitrofurantoin, Ceftazidime, Cefixime and Cefdinir; their resistance to these antibiotics were however, not significantly different from those of Aztreonam, Cefotaxime, Ceftriaxone, and Cefuroxime (p>0.05). Resistance to Norfloxacin, Gentamycin and Amikacin by all the gram negative isolates were not significantly different (p>0.05), but were significantly lower than those of other antibiotics (p<0.05). All the gram negative isolates showed 25 resistance patterns, and all are multi-drug resistant. Resistance to β-lactams antibiotics by both gram positive and gram negative isolates were not significantly different (p>0.05), but were significantly higher than those of other class of antibiotics (p<0.05). Resistance to all the antibiotics by both gram positive and gram negative bacterial isolates were significantly higher than their sensitivity pattern to the same antibiotics.

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**Key words**: sachet water, vehicle, antibiotic, resistance

**1. Introduction**

Many of the routes for the transmission of pathogens are likely to be the same as those for the transmission of antibiotic resistance. These include physical contact, contaminated food and water, body fluids, air-borne inhalation or through vector organisms (Prescott *et al*. 2008). Transmission via contamination of the environment may also occur. Antibiotic resistant organisms in human acquired from these routes mentioned, nosocomially, or as a result of selective pressure on the use of antibiotics, may also be spread into the environment and to other animal species. In addition, antibiotic administered orally to either human or animals may fail to metabolize in the digestive tract. This can result in the antibiotics being excreted in faeces into the environment. These antibiotics may therefore exert their effect on susceptible bacterial population in that environment. If these resistant organisms or faecal material containing resistant organisms contaminate surface or underground water source, they may add to the population of resistant bacteria present in the water body and consequently, the problem of antibiotic resistance. Interactions between normal bacterial populations present in the water source and those added from human or animal source may further increase the burden of antibiotic resistance. The dissemination of antibiotic resistant organisms is influenced by a number of factors such as crowding and unhygienic conditions (Lamikanra *et al*., 1989), inadequate hospital infection control practices (Okeke *e tal*., 1999), economic and political factors (Cornwall, 1997; Dua *et al*., 1994).

Therefore, this study intends to verify sachet water as a potential vehicle for the transmission of antibiotic resistant organisms. This is very significant due to increasing demand for sachet water in Nigeria. Sachet water is readily available and affordable and came into existence due to failure of government to provide portable and affordable water to its populace. More so, sachet water supposingly “treated water” is expected to be pure devoid of any kind of microbial contaminants. There is therefore no justification whatsoever for ‘processed’ water in sachet contaminated with microorganisms of any kind, and their presence even in small numbers render such water being of unacceptable quality or potentially hazardous.

**2. Materials and methods**

**2.1 Sources of samples**

Fifty four sachet water samples from 9 brands were randomly purchased from different sale points in Mubi metropolis, Adamawa State, Nigeria. The names of the 9 brands were coded as follows; MGB, SAN, SHV, CON, SBD, AFM, FAR, ADS and NAF. Each of the brands was replicated six times to have a total of 54 samples.

**2.2 Isolation of organisms**

Standard procedure was employed in the isolation of bacterial isolates from the samples. 10 ml of each sachet water sample was aseptically introduced into 10ml bacteriological peptone waterin test tubes and aerobically incubated at 37oc for 24hrs to encourage bacterial growth. After 24hrs, a loopful of the broth was streak unto McConkey agar and Mannitol salt for differential purposes. All pure isolates from the media were sub-culture into nutrient agar slant, labelled appropriately and refrigerated for further purposes.

**2.3 Identification of the bacterial isolates**

All the isolates were identified appropriately based on standard procedures (Washington *et al*., 2006).

**2.4 The Antibiotic Susceptibility Testing**

The antibiotic susceptibility of the bacterial species isolated was performed on nutrient agar plates by disk diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The commercially available discs (span diagnostic Ltd) containing the following antibiotics were used; Ciprofloxacin (cl,5μg), Chloramphenicol (ck,30μg), Penicillin-G (pg,10μg), Amoxicillin (ax,10μg), Amoxicillin-clavulanic acid (ac,30μg), Cotrimoxazole (ct,25μg), Cephalexin(cp,30μg), Cefazolin (cf,30μg), Cefuroxime (cr,30μg), Erythromycin (er,15μg), Tetracycline (te,30μg), Ofloxacin (of,5μg), Piperacillin (pc,100μg), Azithromycin (az,15μg) for gram positive; and Norfloxacin (nf,10μg), Aztreonam (at,30μg), Cefotaxime (cx,30μg), Ceftriaxone (fr,30μg), Nalixidic acid (na,30μg), Nitrofurantoin (fu,300μg), Cefuroxime (cr,30μg), Gentamycin (gm,10μg), Amikacin (ak,30μg), Ciprofloxacin (cl,5μg), Ofloxacin (of,5μg), Ceftazidime (cz,30μg), Cefixime (fx,5μg), Cefdinir (cn,5μg) for gram negative bacterial isolates. The discs were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 37°C over night. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimetres. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer’s standard zone size interpretative table.

**2.5 Statistical analyses**:

Anova and Student T-test was used to test for significance difference in all the data obtained. All statistical analyses were carried out using the SPSS 17.0 window based program. Significance difference and non- significance difference was defined when p≤ 0.05 and p≥ 0.05 respectively.

**3. Results**:

All the sachet water samples had growth on McConkey agar, while 30(55.5%) of the samples had no growth on Mannitol salt agar. Seventy eight (78) isolates in all were isolated from the 54 sachet water samples. Among the isolates, 24(30.8%) are gram positive, while 54(69.2%) are gram negative. Among the gram positive isolates, coagulase negative staphylococci (CoNS) has the highest incidence rate (70.8%), while Staphylococcus aureus has the least incidence rate (29.2%). Among the gram negative isolates, *Pseudomonas* *aeruginosa* has the highest incidence rate (24.1%), while *Escherichia* *coli* and *Salmonella* spp. has the least incidence rate (9.3% each). On the over all, CoNS has the highest incidence rate (21.8%), while *Escherichia* *coli* and *Salmonella* spp. has the least incidence rate (6.4%) (Table 1).

The results as shown in Table 2 revealed that both *Staphylococcus* *aureus* and CoNS were sensitive to ofloxacin (75%), piperacillin (67%) and ciprofloxacin (67%). Their antibiotic profile however, showed 100% resistant to Penicillin-G, Amoxicillin, Amoxicillin-Clavulanic acid, Cotrimoxazole, Cephalexin, Cefazolin and Cefuroxime; all of which are β-lactams with the exception of Cotrimoxazole. The results showed that resistance by CoNS and *Staphylococcus* *aureus* to chloramphenicol, tetracycline and Azithromycin were not significantly different (p>0.05), but were significantly higher than those of ciprofloxacin, ofloxacin, and piperacillin (p<0.05), and significantly lower than those of other antibiotics (p<0.05). Also, resistance by CoNS and *Staphylococcus* *aureus* to Penicillin-G, Amoxicillin, Amoxicillin-Clavulanic acid, Cotrimoxazole, Cephalexin, Cefazolin, Cefuroxime and Erythromycin were not significantly different (p>0.05), but were significantly higher than those of other antibiotics (p<0.05).

**Table 1: Percentage Occurrence of Bacterial Isolates from Sachet Water**

|  |  |  |  |
| --- | --- | --- | --- |
| **S/N** | **Bacterial isolates** | **Frequency** | **Percentage (%)** |
| 1 | *S. aureus* | 7 | 8.9 |
| 2 | CoNS | 17 | 21.8 |
| 3 | *E. coli* | 5 | 6.4 |
| 4 | *Salmonella spp.* | 5 | 6.4 |
| 5 | *Shigella spp.* | 12 | 15.4 |
| 6 | *P. vulgaris* | 10 | 12.8 |
| 7 | *P. aeruginosa* | 13 | 16.7 |
| 8 | *Citrobacter spp.* | 9 | 11.5 |

**KEY: CoNS=** Coagulase Negative Staphylococci

**Table 2**: **Antibiotic Susceptibility Profile of Gram Positive Isolates**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | **Antibiotics** | ***S. aureus*** | **CoNS** | **Total** |
| **S** | **R** | **S** | **R** | **S** | **R** |
| 1 | Ciprofloxacin | 4(57) | 3(43) | 12(71) | 5(29) | 16(67) | 8(33) |
| 2 | Chloramphenicol | 1(14) | 6(86) | 8(47) | 9(53) | 9(38) | 15(62) |
| 3 | Penicillin-G | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 4 | Amoxicillin | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 5 | Amoxicillin-Clavulanic acid | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 6 | Cotrimoxazole | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 7 | Cephalexin | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 8 | Cefazolin | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 9 | Cefuroxime | 0 | 7(100) | 0 | 17(100) | 0 | 24(100) |
| 10 | Erythromycin | 0 | 7(100) | 1(6) | 16(94) | 1(4) | 23(96) |
| 11 | Tetracycline | 2(29) | 5(71) | 8(47) | 9(53) | 10(42) | 14(58) |
| 12 | Ofloxacin | 4(57) | 3(43) | 14(82) | 3(18) | 18(75) | 6(25) |
| 13 | Piperacillin | 4(57) | 3(43) | 12(71) | 5(29) | 16(67) | 8(33) |
| 14 | Azithromycin | 2(29) | 5(71) | 6(35) | 11(65) | 8(33) | 16(67) |

**TABLE 3:** **Antibiotic Susceptibility Profile of Gram Negative Isolates**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S/N | **Antibiotics** | ***P. aeruginosa*** | ***E. coli*** | ***Salmonella* spp** | ***Shigella* spp** | ***Citrobacter* spp** | ***P. vulgaris*** | **Total** |
| **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** |
| 1 | Norfloxacin | 10(77) | 3(23) | 4(80) | 1(20) | 4(80) | 1(20) | 11(92) | 1(8) | 7(78) | 2(22) | 9(90) | 1(10) | 45(83) | 9(17) |
| 2 | Aztreonam | 0 | 13(100) | 0 | 5(100) | 1(20) | 4(80) | 0 | 12(100) | 0 | 9(100) | 0(9) | 10(100) | 1(2) | 53(98) |
| 3 | Cefotaxime | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 1(10) | 9(90) | 1((2) | 53(98) |
| 4 | Ceftriaxone | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 1(11) | 8(89) | 2(20) | 8(80) | 3(6) | 51(94) |
| 5 | Nalixidic acid | 4(31) | 9(69) | 3(60) | 2(40) | 2(40) | 3(60) | 3(25) | 9(75) | 3(33) | 6(67) | 2(20) | 8(80) | 17(31) | 37(69) |
| 6 | Nitrofuratoin | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 0 | 10(100) | 0 | 54(100) |
| 7 | Cefuroxime | 0 | 13(100) | 1(20) | 4(80) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 0 | 10(100) | 1(2) | 53(98) |
| 8 | Gentamycin | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 9(75) | 3(25) | 7(78) | 2(22) | 9(90) | 1(10) | 48(89) | 6(11) |
| 9 | Amikacin | 8(62) | 5(38) | 5(100) | 0 | 5(100) | 0 | 11(92) | 1(8) | 8(89) | 1(11) | 8(80) | 2(20) | 45(83) | 9(17) |
| 10 | Ciprofloxacin | 6(46) | 7(54) | 3(60) | 2(40) | 1(20) | 4(80) | 5(42) | 7(58) | 6(67) | 3(33) | 6(60) | 4(40) | 27(50) | 27(50) |
| 11 | Ofloxacin | 7(54) | 6(46) | 3(60) | 2(40) | 2(40) | 3(60) | 6(50) | 6(50) | 4(44) | 5(56) | 5(50) | 5(50) | 27(50) | 27(50) |
| 12 | Ceftazidime | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 0 | 10(100) | 0 | 54(100) |
| 13 | Cefixime | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 0 | 10(100) | 0 | 54(100) |
| 14 | Cefdinir | 0 | 13(100) | 0 | 5(100) | 0 | 5(100) | 0 | 12(100) | 0 | 9(100) | 0 | 10(100) | 0 | 54(100) |

**Table 4: Antibiotic Resistance Pattern of *S. aureus* and Coagulase negative Staphylococci (CoNS)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S/N** | Resistance pattern | **No. of isolates** | **No. of antibiotics** | **Designation** |
| **1** | ck,pg,ax, ac,ct, cp, cf, cr,er,pc,az | **3** | **11** | **2sa, 1cons** |
| **2** | ck pg,ax,ac,ct,cp cf,cr,er,te,pc,az | **1** | **12** | **1sa** |
| **3** | ck,cl,pg,ax,ac,ct,cp,cf,cr,te,az | **2** | **11** | **1sa, 1cons** |
| **4** | cl,pg,ax,ac,ct,cp,cf,cr,er,of,pc | **1** | **11** | **1sa** |
| **5** | pg,ax,ac,ct,cp,cf,cr,er,pc | **1** | **9** | **Cons** |
| **6** | ck,pg,ax,ac,ct,cp,cf,cr,er,te,az | **5** | **11** | **1sa, 4cons** |
| **7** | ck,pg,ax,ac,ct,cp,cf,cr,er,te,of,pc,az | **1** | **13** | **Cons** |
| **8** | ck,cl,pg,ax,ac,ct,cp,cf,cr,er,te,of | **1** | **12** | **Cons** |
| **9** | pg,ax,ac,ct,cp,cf,cr,er,of,pc,az | **1** | **11** | **Cons** |
| **10** | cl,pg,ax,ac,ct,cp,cf,cr,er,te,az | **1** | **11** | **Cons** |
| **11** | pg,cl,ax,ac,ct,cp,cf,cr,er,of | **1** | **10** | **Cons** |
| **12** | ck,pg,ax,ac,ct,cp,cf,cr,er,az | **1** | **10** | **Cons** |
| **13** | ck,pg,ax,ac,ct,cp,cf,cr,er,te | **1** | **10** | **Cons** |
| **14** | cl,pg,ax,ac,ct,cp,cf,cr,er | **1** | **9** | **Cons** |
| **15** | cl,pg,ax,ac,ct,cp,cf,cr,er,pc | **1** | **10** | **Cons** |
| **16** | pg,ax,ac,ct,cp,cf,cr,te | **1** | **8** | **Cons** |
| **17** | ck,pg,ax,ac,ct,cp,cf,cr,er,te,of,az | **1** | **12** | **Sa** |

**KEY: sa=** *Staphylococcus**aureus*, **cons**= Coagulase-negative Staphylococcci

**Table 5: Antibiotic Resistance Pattern of Gram Negative Isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **s/n** | **Resistance pattern** | **No. of isolates** | **No. of antibiotic** | **Class of antibiotics** | **Designation** |
| 1 | at,cx,fr,fu,cr,cz,fx,cn | 2 | 8 | 2 | 2cp |
| 2 | at,cx,fr,na,fu,cr,cz,fx,cn | 6 | 9 | 3 | 2pv,1pa,1cp,1sh,1ec |
| 3 | at,cx,fr,fu,cr,of,cz,fx,cn | 2 | 9 | 3 | 1sh, 1ec |
| 4 | at,cx,fr,fu,cr,cl,cz,fx,cn | 2 | 9 | 3 | 1sh, 1ec |
| 5 | at,fr,na,fu,cr,cl,cz,fx,cn | 1 | 9 | 3 | Pv |
| 6 | cx,fr,na,fu,cr,cl,cz,fx,cn | 1 | 9 | 3 | Sal |
| 7 | at,cx,fr,fu,cr,ak,cz,fx,cn | 1 | 9 | 3 | Pa |
| 8 | at,cx,fr,na,fu,cr,of,cz,fx,cn | 6 | 10 | 3 | 2pa,2pv,1sal,1sh |
| 9 | nf,at,cx,fr,fu,cr,of,cz,fx,cn | 2 | 10 | 3 | 1pa,1cp |
| 10 | at,cx,fr,na,fu,cr,gm,cz,fx,cn | 2 | 10 | 4 | 1sh,1cp |
| 11 | at,cx,fr,na,fu,cr,cl,cz,fx,cn | 5 | 10 | 3 | 2pa,2sh,1pv |
| 12 | at,cx,fr,fu,cr,cl,of,cz,fx,cn | 6 | 10 | 3 | 2sal,1pa,1pv,1ec,1h |
| 13 | at,cx,fr,fu,cr,ak,of,cz,fx,cn | 1 | 10 | 4 | 1pv |
| 14 | at,cx,na,fu,cr,ak,cl,cz,fx,cn | 1 | 10 | 4 | 1cp |
| 15 | nf,at,cx,fr,na,fu,cr,cz,fx,cn | 1 | 10 | 3 | 1ec |
| 16 | at,cx,fr,na,fu,cr,ak,cl,cz,fx,cn | 1 | 11 | 4 | 1pa |
| 17 | at,cx,fr,na,fu,cr,gm,of,cz,fx,cn | 1 | 11 | 4 | 1sh |
| 18 | nf,at,cx,fr,na,fu,cr,cl,cz,fx,cn | 1 | 11 | 3 | 1sal |
| 19 | at,cx,fr,na,fu,cr,ak,of,cz,fx,cn | 1 | 11 | 4 | 1pa |
| 20 | at,cx,fr,na,fu,cr,gm,of,cz,fx,cn | 1 | 11 | 4 | 1cp |
| 21 | nf,at,cx,fr,fu,cr,ak,cl,cz,fx,cn | 1 | 11 | 4 | 1pa |
| 22 | at,cx,fr,na,fu,cr,cl,of,cz,fx,cn | 6 | 11 | 3 | 2cp,2sh,1pv,1pa |
| 23 | nf,at,cx,fr,na,fu,cr,gm,ak,cz,fx,cn | 1 | 12 | 4 | 1pv |
| 24 | nf,at,cx,fr,na,fu,cr,ak,cl,cz,fx,cn | 1 | 12 | 4 | 1pa |
| 25 | nf,at,cx,fr,na,fu,cr,gm,ak,cl,cz,fx,cn | 1 | 13 | 4 | 1sh |

**KEY: cp=** *Citrobacter* spp., **pv**= *Proteus* *vulgaris*, **pa**= *Pseudomonas* *aeruginosa*, **sh**= *Shigella* spp., **sal**= *Salmonell* spp., **ec**= *Escherichia* *coli*

The results in Table 3 showed that all the gram negative bacterial isolates were 100% resistance to Nitrofurantoin, Ceftazidime, Cefixime and Cefdinir; their resistance to these antibiotics were however, not significantly different from those of Aztreonam, Cefotaxime, Ceftriaxone, and cefuroxime (p>0.05) (all of which are β-lactam antibiotics with the exception of Nitrofurantoin). Resistance to Norfloxacin, Gentamycin and Amikacin by all the gram negative isolates were not significantly different (p>0.05), but were significantly lower than those of other antibiotics (p<0.05).

Resistance to all the antibiotics by both gram positive and gram negative bacterial isolates were significantly higher than their sensitivity pattern to the same antibiotics. Remarkably, resistance to β-lactams antibiotics by both gram positive and gram negative isolates were not significantly different (p>0.05), but were significantly higher than those of other class of antibiotics (p<0.05).

Seventeen (17) resistance patterns were shown by *Staphylococcus* *aureus* and CoNS and are resistant to 8-13 antibiotics. The most common resistance pattern(ck,pg,ax,ac,ct,cp,cf,cr,er,te,az) has 11 antibiotics exhibited by 4 species of CoNS and 1 species of *Staphylococcus* *aureus* (Table 4).

The results in Table 5 showed that all the gram-negative isolates exhibits 25 resistance patterns out of which all are multi-drug resistant, and are resistant to 8-13 antibiotics. The most common resistant pattern include:

at,cx,fr,na,fu,cr,cz,fx,cn; at,cx,fr,na,fu,cr,cl,of,cz,fx,cn; at,cx,fr,na,fu,cr,of,cz,fx,cn and at,cx,fr,fu,cr,cl,of,cz,fx,cn with six (6) different isolates each.

**4. Discussion**

The findings in this study that CoNS are the most predominant isolates (21.8%) was however contrary to the report of Tagoe *et al.* (2011) in which they showed that *Staphylococcus* *aureus* was the most predominant isolates in their study. *Staphylococcus* *aureus* CoNS isolated from some of the sachet water samples may have entered the water during packaging or handling since the organisms are normal flora of the human skin and nasal cavity (Hunter, 1993). Poor hygienic practices and inadequate sanitary conditions of the production and packaging line account for the major source of microbial contamination of portable water. The presence of *Staphylococcus* *aureus* in sachet water is of public health significance because it is usually responsible for staphylococcal food poisoning (Frazier and Westhoof, 1995), severe soft tissue infections, and toxic shock syndrome (TSS) (Lowy, 1998; Weems, 2001). Coagulase-negative Staphylococci (CoNS) previously dismissed as contaminants are now emerging as important potential pathogens (Garcia *et al*., 2004). In the last two decades, CoNS have also emerged as significant pathogens, especially in immunocompromised patients, premature newborns, urinary tract infections, arthritis, and infections of prosthetic joints (Heikens *et al.*, 2005). The isolation of *Shigella* spp. in this study is also a pointer to poor hygienic practices and inadequate sanitary processes since the organisms can be transmitted via oral-faecal route primarily by faecal contaminated food or water and finger. Isolation of *E. coli* showed faecal pollution of the water samples. Some strains of *E.coli* synthesize heat stable enterotoxins and are responsible for diarrhoeal disease in humans and domestic animals. Isolation of other pathogenic bacteria such as *Proteus* *vulgaris*, *Salmonella* spp., *Pseudomonas* *aeruginosa* *and Citrobacter* spp in sources of drinking water is also of public health significance and similar to the findings of Lateef *et al*. (2005), Olaoye and Onilude, (2009), Tagoe *et al*. (2011), Tula *et al*. (2013). The presence of these organisms may also reflect the poor state of processing methods or facilities; because it is often reported that some unscrupulous producers just bag and seal pipe water without any form of treatment (Nwosu and Ogueke, 2004). There is no justification whatsoever for ‘processed’ water in sachet contaminated with these organisms, and their presence even in small numbers render such water being of unacceptable quality or potentially hazardous.

The result of the antibiotic susceptibility testing showed various percentages of antibiotic resistance among the bacterial isolates from sachet water samples. All the Staphylococcal spp showed 100% resistance to Penicillin-G, Amoxicillin, Amoxicillin-clavulanic acid, Cotrimoxazole, Cephalexin, Cefazolin, Cefuroxime and 96% resistance to Erythromycin. This is a broad spectrum resistance pattern because these antibiotics belong to the class Penicillin, Sulphonamides, Cephalosporin and Macrolide respectively. They were however more susceptible to ciprofloxacin and piperacillin. Earlier study in Ghana by Tagoe *et al*. (2011) observed that most of their isolates from sachet water were resistance to Ampicillin (100%), Penicillin (100%), Cotrimoxazole (57.1%), Cefuroxime (92.9%) and Erythromycin (92.9%).

All theGram negative isolates are 100% resistance to Nitrofurantoin, Ceftazidime, Cefixime and Cefdinir and 98% resistance to Aztreonam, Cefotaxime and Cefuroxime; all of which are cephalosporin with the exception of Nitrofurantoin. Resistance to these antibiotics may reflect the widespread use of these antibiotics. In Nigeria, β-lactams are the most frequently prescribed antibiotics especially in Gram negative infections and selective pressure exerted by the use of these β-lactam drugs have resulted in the strains producing the extended spectrum β-lactamases enzymes (Aibinu *et al*., 2003). Iffat *et al*. (2011), in their work reported high resistance rate among Gram negative organisms against all generations of cephalosporin antibiotics as well as β-lactam/β-lactamase inhibitors. In this study however, 89% gram negative organisms are susceptible to Gentamycin, while 83% are susceptible to Norfloxacin and Amikacin. This is in conformity to a study by Oyetayo *et al*. (2007) who found similar results in *E. coli* isolated from drinking wells in Nigeria. Tagoe *et al*. (2011) also reported 100% sensitivity to gentamycin by all their isolates from sachet water. The high level of resistance to antibiotics is a reflection of misuse and abuse of antibiotics intake in both hospital and environmental settings. Misuse and abuse of antibiotics in these settings may be enhance by over the counter availability of antibiotics, overcrowding and unhygienic conditions, poor regulations on the retails and purchase of antibiotics, ignorance, excessive and inappropriate prescription of antibiotics by clinicians.

The presence of antibiotics resistant bacteria in sachet water is of public health significance because of the danger in promoting multiple antibiotic resistant organisms in humans through possible colonization of the gastrointestinal tract and conjugal transfer of antibiotic resistance to the normal flora leading to more complicated multiple antibiotic resistant organisms (McKeon *et al*., 1995). The prevalence of antibiotic resistant organisms poses a great challenge to clinicians and consumption of water containing these antibiotic resistant organisms may prolong the treatment of not only water-borne diseases (Tagoe *et al*., 2011), but also other infections that may be caused by the resistant organisms or the opportunistic normal flora that acquire resistant genes from these organisms. This implies that treatment of water-borne diseases and other infections caused by these organisms with these antibiotics may be inappropriate and will require new but expensive antibiotics. The high incidence of antibiotic resistant organisms isolated from sachet water samples in this study is worrisome, and need to be addressed properly.

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**Reference**

1. Aibinu I, Odugbemi T and Mee BJ (2003). Extended spectrum β-lactamases in isolates of *Klebsiella* spp. and *Escherichia* *coli* from Lagos, Nigeria. *Nigeria Journal of Health and Biomedical Science.* 2(2): 53-60.
2. Cornwall J (1997). Tuberculosis: a clinical problem of international importance. *Lancet.***350**: 660-661.
3. Dua V, Kunin CM and White LV (1994). The use of antimicrobial drugs in Nagpur, India. A window on medical care in a developing country. *Social* *Science and Medicine*. **38**: 717-724.
4. Frazier WC and Westhoof DC. (1995). Food Microbiology. 4th Edn., Tata McGraw-HillPub. Co. Ltd., New Delhi.
5. Garcia P, Benitez R, Lam M, Salinas AM, Wirth H, Espinoza C, Garay T, Depix MS, Labarca J, Guzman AM (2004). Coagulase-negative *Staphylococci*: clinical, microbiological and molecular features to predict true bacteraemia. *J. Med. Microbiol*. 53: 67-72.
6. Heikens E, Fleer A, Paauw A, Florijn A and Fluit AC. (2005). Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negativ*e* Staphylococci*. J. Clin. Microbiol.* 43(5): 2286-2290.
7. Hunter PR. (1993). The Microbiology of Bottled Natural Water. *J. Applied Bacteriol*., 74: 345-352.
8. Iffat J, hafeez R and Anwar SM (2011). Antibiotic susceptibility pattern of bacterial isolates from patients admitted to a tertiary care hospital in Lahore. *Biomedical*. 27: 19-23.
9. Lamikanra A., Fayinka ST and Olusanya OO (1989). Transfer of low level trimethoprim resistance in faecal isolates obtained from apparently healthy Nigerian students. *FEMS Microbiology Letters.* **50**: 275-278.
10. Lateef, A., oloke, JK. And Gueguimkana, EB. (2005). The prevalence of bacterial resistance in clinical, food, water and some environmental samples in southwest Nigeria. *Environmental* *Monitoring* *and* *Assessment*. 100: 59-69.
11. Lowy FD. (1998). *Staphylococcus aureus* Infections. *New Engl. J. Med.* 339: 520-532.
12. McKeon DM, Calabrae JP and Bissonnette GJ (1995). Antibiotic resistant gram negative Bacteria in Rural ground water supplies. *Wat. Res.*, 29: 1902-1908.
13. National Committee for Clinical laboratory Standards (NCCLS) (2002). Performance standards for antimicrobial susceptibility testing: Twelfth informational supplement. NCCLS document M100-S12. PA, USA.
14. Nwosu JN and Ogueke CC (2004). Evaluation of Sachet Water Samples in Owerri Metropolis. *Nigerian Food Journal.* 22: 164 – 170.
15. Okeke IN, Adebayo L and Robert E (1999). Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. *Emerging Infectious Diseases* **10**: 25: 01.
16. Olaoye OA and Onilude AA (2009). Assessment of microbiological quality of sachet-packaged drinking water in western Nigeria and its public health significance. *Public* *Health*, 123: 729-734
17. Oyetayo VO, Akharaiyi FC and Oghumah M (2007). Antibiotic sensitivity pattern of Escherichia coli isolated from water obtained from wells in Akure metropolis. *Res. J. Microbiol*., 2: 190-193.
18. Prescott LM, Harley JP and Klein DA. (2008). Microbiology. 6th ed., McGraw-Hill, New York. pp. 1085.
19. Tagoe DNA, Nyarko H, Arthur SA and Birikorang E (2011). A study of antibiotic susceptibility pattern of bacteria isolates in sachet drinking water sold in the cape coast metropolis of Ghana. *Research Journal of Microbiology*. 10:1-6
20. Tula MY, Iruolaje FO, Bitrus J, Iliyasu AJ and Jennifer HH (2013). Microbiological Perspective on the Quality and Safety of Borehole Water in Mubi Metropolis, Nigeria. World Rural Observ. 5(4): 1-6.
21. Washington CW, Stephen DA, Williams MJ, Elomer WK., Gary WP, Paul CS and Gail LW (2006). Koneman’s Colour Atlas and Textbook of Diagnostic Microbiology, 6th edn. Lippincott Williams and Wilkins, Philadelphia, USA.
22. Weems JJ (2001). The many faces of *Staphylococcus aureus* infection. *Postgraduate Med.* 110 (4):24-36.

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