Antimicrobial Resistance of Enterobacteria to Some Commonly used Antibiotics in General Hospital Akwanga, Nasarawa State, Nigeria

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Abstract: ***Antibiotic resistance is now generally accepted as a major public health issue. This work sought to determine the antibiotic resistant profile of some members of the Enterobacteriaceae family to some commonly used antibiotics. One hundred and twenty (120) clinical isolates of Escherichia coli (40), Klebsiella spp (40) and Salmonella spp (40) were collected from General Hospital Akwanga, Nasarawa State, Nigeria and were tested against Ciprofloxacin, Streptomycin, Erythromycin, Sulphamethoxazole/Trimethoprim, Ampicillin , Tarivid, Cefoxime, Gentamicin, Augmentin and Nalidixic acid, using the Agar disc diffusion technique. The results revealed 57.5%, 54%, and 49.25% instances of resistance to Escherichia coli, Klebsiella spp and Proteus spp respectively to the tested antibiotics. The high resistance recorded is in agreement with the findings of some other researchers. Findings from this work also show the high susceptibility of the Enterobacterial isolates to Ciprofloxacin (Floroquinolone) antibiotic. The result suggests that the use of the Fluoroqunolones in the treatment of the infections caused by these organisms should be encouraged. Also Search for novel antibiotics that would interfere with the genetic mechanism of microbial cells should be encouraged.***

Keywords: Antibiotics, Enterobacteria, Floroquinolone and Resistance.

1. Introduction

Gram-negative rods belonging to the family Enterobacteriaceae are common agents of infections in hospitalized patients. They cause a large proportion of the cases of community and hospital-acquired bacteraemia [1]-[3] and majority of cases of hospital-acquired pneumonia (Schaberg et al., 1991), both being severe infections associated with a high mortality [5], [6]. Antibiotic resistance of bacteria is biological risk, which increases morbidity and mortality of animals and humans [7]. In recent years accumulating problems with bacteria that are resistant to antibiotics occur. It is leading them to predictions that we return to the time before the discovery of antibiotics [8].

The distribution of Enterobacteriaceae infections are worldwide [9], with more occurrences in developing countries. Medical efforts through chemotherapy have helped in providing treatment for infections caused by the members of the Enterobacteriaceae but most of them are increasingly becoming resistant to several antimicrobial agents [10]. Decreased susceptibility of most members of the Enterobacteriaceae family to most antibiotics as passed serious threat to public health. The emergence and dissemination of antimicrobial resistance among pathogens is one of most serious threat to the successful treatment of microbial diseases and this has generated worldwide concern in medical community especially in developing countries[22], [12]-[14].

Antibiotic resistance is now generally accepted as a major public health issue. High frequencies of antimicrobial resistance have been found in enterobacteria, in faecal flora in clinical isolates [15]. Members of the family *Enterobacteriaceae* have a worldwide distribution and are found in various environments and hosts such as water, soil, plants, human beings, and other animals, but they are also significant causes of human diseases. Antibiotic resistance among them has been regularly monitored in the hospital in some developed countries [16], [17]. In contrast, there is still very scarce information of enterobacterial resistance to antimicrobial use by humans in Nigeria.

Although Enterobacteriaceae infections occurs worldwide [9], some regions of the world have high incidence of the infections. For instance Klebsiella rhinoscleromatis which causes granulomatis is found mainly in central and South America, Morocco and parts of Eastern Europe. Enterohaemorrhagic E. coli infections i.e. life threatening haemorrhagic diarrhoea or colitis has been reported mainly from America and Europe. Recent plague reports have come from many parts of Africa, Asia, Europe and America (Cheesbrough, 2000). Infections caused by extended spectrum beta-Lactamase producing gram-negative bacilli (majorly enterobacteria) constitute a growing worldwide problem [9]. For instance, there are 164.7 million cases of Shigellosis throughout the world of which 163.2 million occur in developing countries with 1.1 million deaths (61% involving children under five years) [18].

Since the outcome of severe infections caused by

Enterobacteria may depend on rapid and appropriate therapy, and the choice of antibiotic is often based on the knowledge of the susceptibility profiles of the bacteria most commonly encountered [19]. This study was conducted to evaluate the susceptibility of the enterobacteria isolated from clinical samples to some commonly used antibiotics, and to determine the antimicrobial resistance pattern of those isolates.

2. Materials and Method

2.1 Samples Collection

For this study, 120 pure clinical isolates of Enterobacteria were obtained from the Laboratory section of General Hospital Akwanga, Nasarawa State, Nigeria between June to November, 2012. These isolates consisted of the following Enterobacterial species: Escherichia coli (40 isolates), Klebsiella spp (40 isolates) and Salmonella spp (40 isolates).

2.2 Antimicrobial Susceptibility Test

The isolates were screened for antimicrobial susceptibility, using the agar disk diffusion method [20]. The following antibiotics (Oxoid) were used: Augmentin (10 µg), Gentimicin (10µg), Ciprofloxacin (5µg), Streptomycin (10µg), Nalidixic acid (30µg), Tarivid (30µg), Cefotaxime (30µg), Ampicillin (10µg), Erythromycin (5µg) and Sulphamethoxazole/Trimethoprim (25µg).

The isolates were uniformly streaked on Muller-Hinton agar plate and the antibiotic impregnated discs were applied onto the inoculated plates using sterile forceps. The plates were then incubated at 37oC for 24hrs, after which clear zones of inhibition for each antibiotic were measured using transparent ruler. The results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria [21].

3. Results

The results of the in vitro activity of the antibiotics against the tested enterobacterial isolates are as presented in Tables 1 and 2. These results show that 26 (65%) of the 40 E. coli isolates were sensitive to Ciprofloxacin, 21 (52.5%) were sensitive to Streptomycin, 24 (60%) were sensitive to Erythromycin, 19(47.5%) were sensitive to Sulphamethoxazole/Trimethoprim, 22 (55%) were sensitive to Tarivid, 14 (35%) were sensitive to Cefotaxime, 15(37.5%) were sensitive to Gentimicin, 13(32.5%) were sensitive to Augmentin, while 8 (20%) were sensitive to Ampicillin and Nalidixic acid (Table 1).

However, 32 (80%) of the 40 E. coli isolates were resistant to Ampicillin and Nalidixic acid, 27 (67.5%) were resistant to Augmentin, 26 (65%) were resistant to Cefotaxime, 25 (62.5%) were resistant to Gentamicin, 21 (52.5%) were resistant to Sulphamethoxazole/Trimethoprim, 19 (47.5%) were resistant to Streptomycin, 18 (45%) were resistant to Tarivid, 16 (40%) and 14 (35%) were resistant to Erythromycin and Ciprofloxacin respectively (Table 2).

Out of the 40 Klebsiella spp tested, 28 (70%) were sensitive to Ciprofloxacin, 26 (65%) were sensitive to Erythromycin, 25 (62.5%) were sensitive to Streptomycin, 24 (60%) were sensitive to Tarivid, 22 (55%) were sensitive to Gentamicin, 15 (37.5%) were sensitive to Augmentin, 14 (35%) were sensitive to Cefotaxime, 12 (30%) were sensitive to Sulphamethoxazole/Trimethoprim and Nalidixic acid, 6 (15%) were sensitive to Ampicillin (Table 1).

More so, 34 (85%) of the 40 Klebsiella spp isolates were resistant to Ampicillin, 28 (70%) were resistant Sulphamethoxazole/Trimethoprim and Nalidixic acid, 26 (65%) were resistant to Cefotaxime, 25 (62.5%) were resistant to Augmentin, 18 (45%) were resistant to Gentamicin, 16 (40%) were resistant to Tarivid, 15 (37.5%) were resistant to Streptomycin, 14 (35%) were resistant to Erythromycin, and 12 (30%) were resistant to Ciprofloxacin (table 2).

Out of the 40 Proteus spp isolates tested, 32 (80%) were sensitive to Ciprofloxacin, 30 (75%) were sensitive to Streptomycin, 29 (72.5%) were sensitive to Erythromycin and Tarivid, 20 (50%) were sensitive to Gentamicin, 18 (45%) were sensitive to Augmentin, 17 (42.5%) were sensitive to Sulphamethoxazole/Trimethoprim, 11 (27.5%) were sensitive to Nalidixic acid, 10 (25%) were sensitive to Ampicillin, and 7 (17.5%) were sensitive to Cefotaxime (Table 1).

However, 33 (82.5%) of the 40 Proteus spp isolates tested were resistant to Cefotaxime, 30 (75%) were resistant to Ampicillin, 29 (72.5%) were resistant to Nalidixic acid, 23 (57.5%) were resistant to Sulphamethoxazole/Trimethoprim, 22 (55%) were resistant to Augmentin, 20 (50%) were resistant to Gentamicin, 11 (27.5%) were resistant to Erythromycin and Tarivid, 10 (25%) were resistant to Streptomycin and 8 (20%) were resistant to Ciprofloxacin (Table 2).

Table 1: Invitro Antimicrobial Susceptibility Pattern of Enterobacteria Isolates to Some Commonly used Antibiotics General Hospital Akwanga, Nigeria

	Percentage (%) Sensitivity to Antimicrobial Agent										
Test Organism	Total tested	Cipr (%)	Stre (%)	Eryt (%)	Sulp (%)	Ampi (%)	Tariv (%)	Cefo (%)	Genta (%)	Augm (%)	Nali (%)	Total % Susceptibility
Escherichia coli	40	26(65)	21(52.5)	24(60)	19(47.5)	8(20)	22(55)	14(35)	15(37.5)	13(32.5)	8(20)	42.5
Klebsiella spp	40	28(70)	25(62.5)	26(65)	12(30)	6(15)	24(60)	14(35)	22(55)	15(37.5)	12(30)	46
Proteus spp	40	32(80)	30(72)	29(72.5)	17(42.5)	10(25)	29(72.5)	7(17.5)	20(50)	18(45)	11(27.5)	50.75

Legend: Stre=Streptomycin, Eryt =Erythromycin, Sulp= Sulphamethoxazole/Trimethoprim, Ampi=Ampicillin, Tari=Tarivid, Cefo=Cefoxime, Gent=Gentamicin, Augm=Augmentin, Nali=Nalidixic, Cipr=Ciprofloxacin

Table 2: Invitro Antimicrobial Resistance Pattern of Enterobacteria Isolates to Some Commonly used Antibiotics in General Hospital Akwanga, Nigeria

	Percentage Resistivity to Antimicrobial Agent										
Test Organism	Total tested	Cipr (%)	Stre (%)	Eryt (%)	Sulp (%)	Ampi (%)	Tari (%)	Cefo (%)	Gent (%)	Augm (%)	Nali (%)	Total % Susceptibility
Escherichia coli	40	14(35)	19(47.5)	16(40)	21(52.5)	32(80)	18(45)	26(65)	25(62.5)	27(67.5)	32(80)	57.5
Klebsiella spp	40	12(30)	15(37.5)	14(35)	28(70)	34(85)	16(40)	26(65)	18(45)	25(62.5)	28(70)	54
Proteus spp	40	8(20)	10(25)	11(27.5)	23(57.5)	30(75)	11(27.5)	33(82.5)	20(50)	22(55)	29(72.5)	49.25

Legend: Stre=Streptomycin, Eryt =Erythromycin, Sulp= Sulphamethoxazole/Trimethoprim, Ampi=Ampicillin, Tari=Tarivid, Cefo=Cefoxime, Gent=Gentamicin, Augm=Augmentin, Nali=Nalidixic, Cipr=Ciprofloxacin

4. Discussion

Antimicrobial resistance among several members of the family Enterobacteriaceae has been recognized worldwide as a serious threat to humanity in medical community, especially in developing countries and this has complicated treatments of microbial diseases [12], [13]. The pattern of resistance to these antimicrobial agents has also been well documented [22]. The results presented in this work are also in agreement to the previous documentation.

Most of the 120 Enterobacterial isolates examined displayed resistance to one or more antibiotics in different percentages. This is in line with previous reports which suggested that the unwarranted use of some of these antibiotics has been a key factor in the emergence of antimicrobial resistance [12].

The highest resistance was recorded in E. coli, with 57.5% of the isolates being resistant to the antibiotics, where resistance to Ampicillin and Nalidixic acid were the highest. The multi-resistance of E. coli isolates to most of the antibiotics used in the present study has been reported by several investigators [23], [3]. The high resistance observed among E. coli isolates could be as a result of their wide spread urinary tract infections with various strains which have been exposed to most of the commonly used antibiotics. This correlates with the previous work carried by Schroeder et al. [12], who reported high resistance among E. coli isolates.

About 54% of Klebsiella spp isolates tested were resistant to the antibiotics, with approximately all displaying multiple resistances. This may have been because of the wide range of nosocomial and respiratory tract infections caused by some Klebsiella spp. These results are in agreement with those isolates reported by several authors [24], [25], [2].

The lowest resistance recorded among the Enterobacterial isolates tested was found in Proteus spp with 49.25% resistance. Among the isolates of Proteus spp tested, resistance was very high to Ampicillin. This may be due to the frequent exposure as well as patients' attitude towards these antibiotics. For these commonly used antibiotics, resistance of the members of Enterobacteriaceae family tested is in the order E. coli > Klebsiella spp> Proteus spp.

Resistance to Nalidixic acid, Ampicillin, and Gentamicin was expected to be very low, as these antibiotics are effective against the Enterobacteriaceaea. However, significant instances of resistance were shown to these antibiotics. This could probably be due to frequent usage of these antibiotics.

Ciprofloxacin was found to be highly effective in all the isolates tested. This shows the effectiveness of the Fluoroquinolones. This is in agreement with the finding of Scheld [13], suggesting the use of this class of antibiotics against infections caused by Enterobacteriaceae members. Ciprofloxacin is a potent broad spectrum antibacterial agent. Prior to its use, resistance was rare [26]. Egri-Okwaji et al. [27] also reported 100% susceptibility of E. coli isolates to Ofloxacin while Kesah et al. [28] recorded a 2% resistance of E.coli to fluoroquinolone. A previous assessment of some six antibiotics against clinical isolates of E. coli showed Ciprofloxacin to be the most efficacious [30]. However some workers have recently reported resistance to the quinolones in Nigeria as well as in other countries [29], [30].

Resistance was found to be relatively low to Streptomycin. This is probably because of less exposure to the antibiotic due to the discouraged use of the antibiotic and the fact that it is usually administered intravenously thereby restricting indiscriminate use [18]. This shows that Streptomycin can be used as an antibiotic of choice against Enterobacteriaceae infections, except for its serious side effect [14]. Although Gentamicin is a narrow spectrum antibiotic against gram-negative pathogens, yet significant resistance was noticed. This may be due to the frequent prescription of this antibiotic which has greatly decreased its usefulness.

The multiple resistances observed in this study may have been from the spread of mobile genetic elements, since most of the isolates resistant to a particular antibiotic were found to be resistant in another. This has been suggested by Schroeder et al. [12]. Some of the enterobacterial isolates tested exhibited intermediate resistance. The antibiotics that showed such characteristic may likely respond to treatment if the concentration of the antibiotic is increased. Larger doses are used or the antibiotic is concentrated at the site of infections.

References

[1] H.F. Geerdes, D. Ziegler, H. Lode, M. Hund, A. Loehr, W. Fanfmann, J. Wagner, "Septicemia in 980 patients at a university hospital in Berlin: Prospective studies during 4 selected years between 1979 and 1989," Clinical Infectious Diseases Journal, 15, pp. 991-1002, 1992.

[2] A.H. Al-Charrakh, "Antibiotic resistance of Klebsiella, Enterobacter, and Serratia strains isolated from clinical specimens in Najaf. Iraq" Journal of Babylon University, 5(4), pp. 478-482, 2000.

[3] A.H. Al-Charrakh, A.M. Al-Muhana, Z.H. Al-Saadi, "Bacterial profile of blood stream infections in children less than three years old," Journal of Babylon University, 10 (3), pp. 481-485, 2005.

[4] D.R. Schaberg, D.H. Culver, R.P. Gaynes, "Major trends in the microbial etiology of nosocomial infection," American Journal of Medicine, 91, pp. 72S–75S, 1991.

[5] J. Davies, "Origins and evolution of antibiotic resistance," Microbiologia, 12, pp. 6-19, 1996.

[6] E. Lautenbach, B. Strom, W. Bilker, I. Patel, P. Edelstaic, N. Fishman, "Epidemiology Investigation of

Flouroquinolones Resistance in Infections Due to Extended–Spectrum Beta-Lactamase Producing E. coli and Klebsiella pneumonia," Clinical Infectious Diseases Journal. 33(8), pp. 1288, 2001.
[7] EFSA. "Foodborne antimicrobial resistance as a biological hazard, Draft Scientific Opinion of the Panel on Biological Hazards (Question No EFSA– Q-2007-089)," Draft endorsed on 6 March, 2008.
[8] P. Keyser, M. Elofson, S. Rosell, H. Wolf-Watz, "Virulence blockers as alternatives to antibiotics: type III secretion inhibitors against Gram-negative bacteria," Journal of International Medicine, 264, pp. 17-29, 2008.
[9] Z. Kanafani, A. Mehio-sibai, G. Aral, M. Kanaan, S. Kanji, "Epidemiology and Risk Factors for Extended Spectrum Beta-Lactamase Producing Organism: A Case Control Study Of a Tertiary Health Care Centre In Behavior," Journal of Infection Control, 33, pp 326-332, 2005.
[10] E. Threfall, A. Graham, T. Cheasty, R. Ward, B. Rowe, "Resistant to Ciproflocxacin in Pathogenic Enterobacteriaceae in England and Wales in 1996," Journal of Clinical Pathology. 50(12), pp. 1027-1028, 1997.
[11] S.E. Agina, S.S. Oyelola, T.O.E. Ekpedendu, K. Ibrahim, "Evaluation of the resistance some pathogenic bacteria to commonly stock/vented antibiotics," Nigerian Journal of Biotechnology, 11, pp. 9–13, 2000.
[12] C.M. Schroeder, J.S. Mena, C. Debroy, J. Tarcolin, C. Zhao, F.P. Dercott, D.D. Wangnes, D.R. Walke, G.D. White, "Antimicrobial Resistant of E. coli 026,0103, 0111, 0238 and 0145 From Animals and Humans," Emerging Infectious Disease Journal, 8(12), pp. 1409-1412, 2002.
[13] M.W. Scheld, "Maintaining Fluroquinolone Class Efficiency: Review of Influencing Factors," Emerging Infectious Disease Journal, 9(1), pp. 1-9, 2003.
[14] M.L. Prescott, P.J. Harley, A.D. Klein, "Microbiology" 6th edition.Mc Graw Hill Publishers, New York, pp 78-793, 2005.
[15] T. Leistevuo, J. Leistevuo, M. Österblad, T. Arvola, P. Toivonen, T. Klaukka, A. Lehtonen, P. Huovinen, "Antimicrobial resistance of fecal aerobic gram-negative bacilli in different age groups in a community," Journal for Antimicrobial Agents Chemotherapy, 40, pp. 1931-1934, 1996.
[16] B. Alex, G. Wil, V. Cindy, L. Nicile, C. Margreet, C. Jan, L. Elly, M. Siem, V. Henri, L. Bob, E. Hubert, "Re-emergence of Ciprofloxacin-Resistance Enterobacteriaceae Containing Multiple Gentamicin Resistance-Associated lntergrons, the Netherlands," Emerging Infections Diseases.5, pp. 1-11, 2001.
[17] J. Amita, R. Indranil, K. Mahendra, K. Mala, "Prevalence of Extended Beta-Lactamase Producing Gram-negative Bacteria in Septicaemase Neonates in Tertiary Care Hospital," Journal of Medical Microbiology. 52, pp. 421-425, 2003.
[18] M. Cheesbrough, "District Laboratory Practice in Tropical Countries," Cambidge Low-Price Editions, Part 2. pp. 132-199, 2000.
[19] A. Alaa, S.A. Murooj, K.A. Wilad, "Antibiotic Susceptibility of EnterobacteriaIsolated from One Hospital in Hilla, Iraq," Journal of Babylon University/Pure and Applied Sciences, 1(19), pp. 36-44, 2011.
[20] W.M.M. Kirby, A.W. Bauer, J.C. Sherries, M. Turck, "Antibiotics Susceptibility Testing," American Journal of Pathology. 45, pp. 493-496, 1966.
[21] CLSI, "Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth InformationalSupplement," M100-S15; Clinical and Laboratory Standards Institute Wayne (CLSI): Chicago, IL, USA; Volume 25, No. 1, 2007.
[22] S.E. Agina, S.S. Oyelola, T.O.E. Ekpedendu, K. Ibrahim, "Evaluation of the Resistance of some Pathogenic Bacteria to Stock/Vended antibiotics," Nigerian Journal of Biotechnology, 11, pp. 9-13, 2000.
[23] I.C. Okoli, "Antimicrobial Resistance Profiles of E. coli Isolated From Free Range Chickens in Urban and Rural Environments of Imo State Nigeria," Journal of Health Allied Sciences, 1, pp. 1-11, 2006.
[24] J. Sirot, C. Chanal, A. Petit, D. Sirot, R. Labia, G. Gerbaud, "Klebsiella pneumoniae and other Enterobacteriaceae producing novel plasmid-mediated beta-lactamases markedly active against third-generation cephalosporins: epidemiologic studies," Rev. Infect. Dis., 10, pp. 850–859, 1998.
[25] K.S. Thomson, C.C. Sanders, J.A. Washington, "High-level resistance to cefotaxime and ceftazidime in Klebsiella pneumoniae isolates from Cleveland, Ohio," Antimicrob. Agents Chemother. 35, pp. 1001-1005, 1991.
[26] A.L. Barry, P.C. Fuchs, M.A. Pfaller, S.D. Allen, E.H. Gerlach, "Prevalence of fluoroquinolone resistant bacterial isolates in four medical centers during the first quarter of 1990," Euro J Clin Micro Infect Dis. 9, pp. 906–8, 1990.
[27] M.T.C. Egri-Okwaji, E.O. Iroha, C.N. Kesah, T. Odugbemi, "Bacterial pathogens causing neonatal sepsis in an out-born neonate unit in Lagos, Nigeria," Nig Qtr J Hosp Med. 6, pp. 149–152, 1996.
[28] P.I. Umolu, O. Omigie, Y. Tatfeng, F.I. Omorogbe, F. Aisabokhale, O.P. Ugbodagah, "Antimicrobial susceptibility and plasmid profiles of Escherichia coli isolates obtained from different human clinical specimens in Lagos – Nigeria," Jour Am Sci. 2(4), pp. 70-76, 2006.
[29] A. Daini, D. Ogbodu, O. Ogunledun, "Quinolones Resistance and R-Plasmids of Some Gram-Negative Enteric Bacilli," African Journal of Clinical and Experimental Microbiology, 11, pp. 12-14, 2005.
[30] J. Oteo, E. Lazaro, F.J. DeAbjo, F. Baquero, J. Campos, "Spanish members of EARSS. Antimicrobial - resistant invasive Escherichia coli, Spain," Emerg Infect Dis. 11 (4), pp 546-553, 2005

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