Original research article

Bacterial resistance to commonly prescribed antibiotics in a tertiary care hospital: a retrospective review of evidence

Abstracts

Background: Antibiotic resistance is a growing global healthcare challenge and efforts to contain it are being outpaced by rapid emergence of resistant microbes. Common environmental pathogens have been reported to be manifesting resistance to several antibiotics to which they were once sensitive. In hospital setting the close contact between patients and caregivers allow resistant strains to easily spread in the wards. Evidence of antibiotic resistance is needed to inform rational selection of drugs for infectious diseases.

Aim: The aims of this study were to determine common pathogenic bacterial isolates among patients and their antibiotic sensitivity profiles.

Methods: This was a cross sectional retrospective study using laboratory records of antibiotic resistance profiles of bacterial isolates obtained from patients. Antibiogram records for one year period were obtained and relevant data extracted for analysis

Results/Discussion: The most commonly isolated bacteria included *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* which together accounted for over two thirds of isolates. Most of the bacterial isolates were resistant to at least four antibiotics with Penicillins, Sulphonamides and Tetracyclines having the highest level of resistance. Resistance to these antibiotics is well reported in literature and their empirical prescription threatens their efficacy in treatment of infectious diseases.

Conclusion: Antibiotic resistance among isolates of pathogenic bacteria is high and there is need to emphasize evidence based rational prescriptions to not only improve clinical outcomes but also to preserve the efficacy of current antibiotic stock.

Introduction: Antimicrobial resistance is a rising global problem particularly in low to middle income countries where regulatory controls and health care systems are weak. A combination of high infectious disease burden, poor access to quality medicines and diagnostic services as well as low availability of second line antibiotics all combine to increase morbidity and mortality [WHO 2012, WHO 2004, Frean *et al*, 2012]. Antimicrobial resistance is widespread and it's now threatening future public health and making infectious diseases difficult to manage. In recent years emerging evidence have shown that irrational use of antibiotics in both animals and humans is accelerating the development of multiple microbial resistance to commonly prescribed antibiotics particularly in low income countries [Levy *et al*, 2004, Lateef 2004, Nsofor *et al*, 2012, Ohi *et al*, 2011].

In many developing countries where a combination of weak healthcare systems and poor regulatory controls allow antibiotics to be freely available without medical prescription coupled with irrational prescription and misuse of antibiotics contribute to emergence of resistant bacterial strains [Lim *et al*, 2015, Zimmer 2015]. In Nigeria, several studies have reported high level of irrational prescription of antibiotics in healthcare facilities [Umar *et al*, 2018, Eshiet *et al*, 2015, Akinyandenu *et al*, 2014, Oduyebo *et al*, 2017]. While prevalence may

vary widely between countries, frivolous antibiotic prescription is a global problem [Kourlaba *et al*, 2016, Akram *et al*, 2014, Kaur *et al*, 2018].

Antibiotic resistance to antibiotics is well documented particularly among five common bacterial pathogens [*Staphylococcus aureus*, *Escherichia coli*, *Proteus spp*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, alpha *Haemolytic streptococci* etc.]. In China, resistance of *Escherichia coli* to Quinolones is in the range of 53 – 56% and 31 – 70% with third generation Cephalosporins. Resistance of *Klebsiella pneumoniae* is reported to be between 25 – 52% [Cui *et al*, 2017].

In sub Saharan Africa, some studies reported resistance to Ampicillin by *Escherichia coli* and *Klebsiella pneumonia* averaged 75.4% and 97% of strains respectively; a third of these organisms are reported to be resistant to Amoxicillin + Clavulanic acid [Adjei *et al*, 2012, Oli *et al*, 2010, Oladeinde *et al*, 2011, Muoneke *et al*, 2012, Rabasa et al 2002, Okwori *et al*, 2010, Mava *et al*, 2012, Sire *et al*, 2007]. It was reported that *Escherichia coli* is a to be the leading cause of urinary tract infections globally [Stamm *et al*, 2001, Russo *et al*, 2003] which often result in increased morbidity and mortality [Dehbanipour *et al*, 2016].

Resistance to Quinolones by many pathogenic bacteria is becoming a major concern among clinicians globally [Pitout *et al*, 2008, Urban *et al*, 2010, Amabile-Cuevas *et al*, 2010, Silva-Sanchez *et al*, 2013, Paniagua-Contreras *et al*, 2017, CDDEP 2015]. Prevalence of Methicillin resistant *Staphylococcus aureus* [MRSA] varies widely between countries and sometimes between various departments of the same hospital [Robicsick *et al*, 2008, Gordon *et al*, 2008, Jarvis *et al*, 2007, Haznedaroglu *et al*, 2010, Ramirez-Castillo et al, 2018] and while its occurrence is decreasing in developed countries because of sustained action, the reverse is the case in many developing countries.

The emergence of multidrug resistant strains of *Pseudomonas aeruginosa*, *Klebsiella* spp and MRSA in hospital settings is well reported in literature {Rice 2006, Misic *et al*, 2014, Iredell *et al*, 2016]. For instance resistance of gram negative bacterial isolates to Aminoglycosides and Quinolones is reported to have increased in recent years [Bubonja-Sonje *et al*, 2015, Labarca *et al*, 2016]. While prevalence is highly variable, there is consistent evidence to conclude that high levels of resistance of both gram positive and negative bacteria pose significant risks to public health [Nsofor *et al*, 2016, Jombo *et al*, 2011,, Muluye *et al*, 2014, Ruiz *et al*, 2016, Trojan *et al*, 2016, CDDEP 2015].

Multidrug resistance above 50% have been reported with many bacterial strains in many sub Saharan African countries [Kariuki *et al*, 2015]. In one study, it was observed that 84% of *Klebsiella pneumonia* strains were resistant to Cephalosporins; about 47% of Enterobacteriacea isolates were resistant to third generation Cephalosporins and 31 – 94% of isolates were resistant to Chloramphenicol [Le Daore *et al*, 2014]

Overall, high rates of resistance of gram positive pathogens in hospital acquired infections are reported to be highly resistant to first line antibiotics [Leopold *et al*, 2014]. Evidence of high level resistance to commonly prescribed antibiotics is yet to significantly influence treatment guidelines of many common invasive bacterial infections. The impact of rising antimicrobial resistance due to empirical antibiotic prescription practices is yet to be widely evaluated in healthcare facilities in low income countries including Nigeria. The cost of microbial resistance and impact on patient clinical outcomes has also received little research attention in low income countries [Blomberg *et al*, 2005]. Microbial sensitivity to antibiotics has changed and evidence from healthcare facilities can provide valuable insight into trends, spread and severity. This will help in formulating antibiotic use

guidelines in hospitals and the evidence to guide cautious use of broad spectrum and new generation antibiotics.

Objectives: The aims of this study are to determine the level of microbial resistance to commonly prescribed antibiotics and investigate prevalence of pathogenic bacteria in laboratory samples

Methods

Setting: The study was carried out in the microbiology department of the University of Maiduguri teaching hospital, Borno State Nigeria.

Study design: This was a cross sectional retrospective study using microbial sensitivity test records in the Microbiology laboratory of the hospital.

Data collection: Records of bacterial isolates from all patient samples and their sensitivity/resistance results were extracted into data collection forms. Isolates were from Urine, Blood, Sputum, swab [HVS, wound and pus]. Antibiogram followed standard test procedures using antibiotic impregnated test discs in appropriate growth medium. The level of bacterial resistance to each antibiotic was demonstrated by zones of inhibition of bacterial growth.

Data analysis: The data was entered into SPSS 20 for descriptive analysis. Results were expressed as percentages and average.

Ethical approval: It was received from human ethics research committee of University of Maiduguri teaching hospital.

Results: Demographic data indicated that females were the majority of patients from where over two thirds of samples were obtained [67.2%] and males represented about a third of the population [Figure 1]

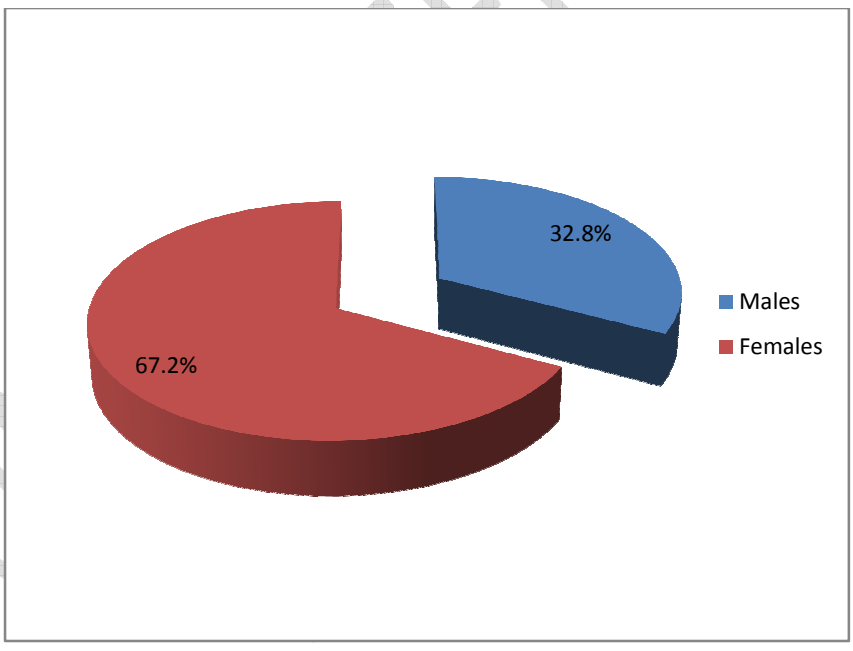

Figure 1: Gender distribution

Age distribution showed that majority of patients was below 50 years [81.4%] of which those within 21 – 30 year old bracket represented the largest age group [Figure 2].

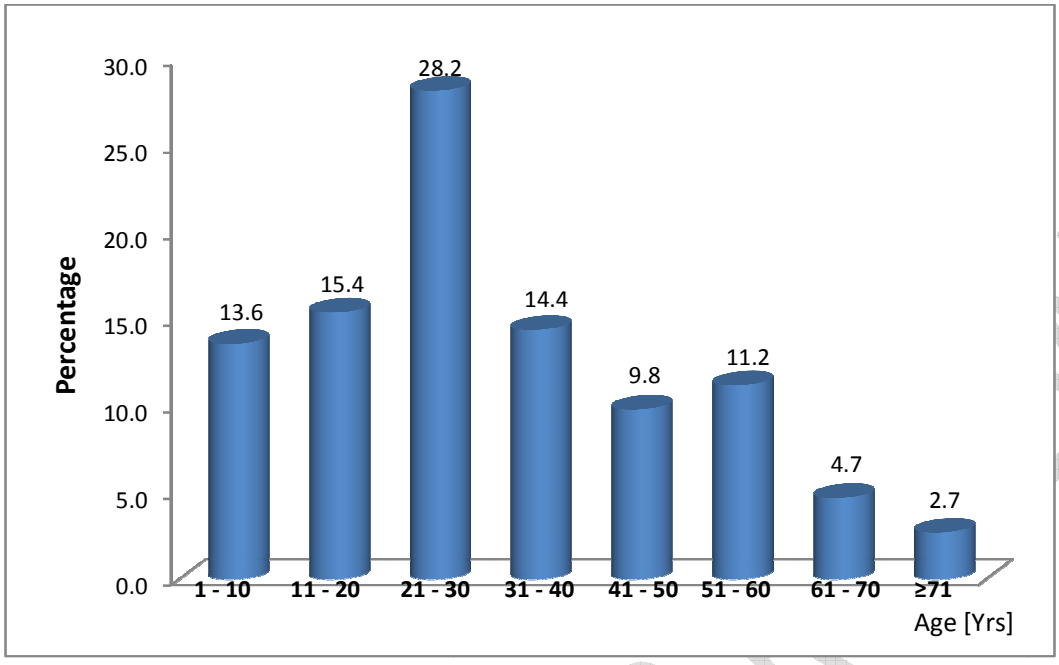

Figure 2: Age distribution

A total of 4691 laboratory samples were tested for the presence of pathogenic bacteria of which 829 [17.6%] returned with positive results. Distribution showed that Sputum samples returned with the most positive results [57.1%] compared with blood samples which had about 6.7% positive result [Figure 3]

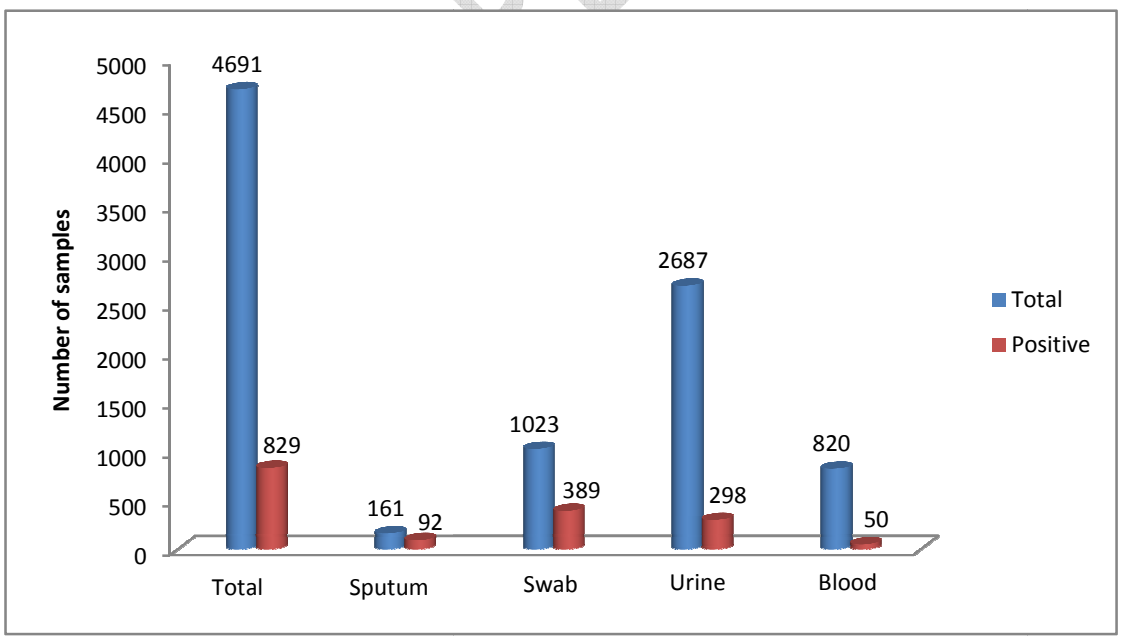

Figure 3: Distribution of pathogenic bacterial isolates among clinical samples

The most common pathogenic bacterial isolates were *Staphylococcus aureus* [37.3%], *Escherichia coli* [22.7%] and *Klebsiella* pneumoniae [13.1%]. These bacteria were found in all laboratory samples obtained from patients compared with *Proteus* species, *Pseudomonas aeruginosa* and coliforms which were largely found in urine and swab samples [Table 1]

Specimen	SA	EC	KB	PT	PS	HS	CF
Swab	244	64	58	27	29	7	36
Urine	23	114	36	8	10	--	52
Sputum 10	3	9	--	4	39	4	
Blood	32	7	6	--	--	--	7
Total	309	188	109	35	43	46	99

Key: *SA = Staph aureus, EC = E.Coli, KP = Klebsiella pneumoniae, PT = Proteus spp, PS =Pseudomonas aeruginosa, Haemolytic streptococci HS, CF = Coliforms*

The overall prevalence of pathogenic bacteria in all clinical isolates showed that *Staphylococcus aureus* accounted for over a third of pathogenic bacteria while *Proteus* species were the least commonly encountered isolate [Figure 4].

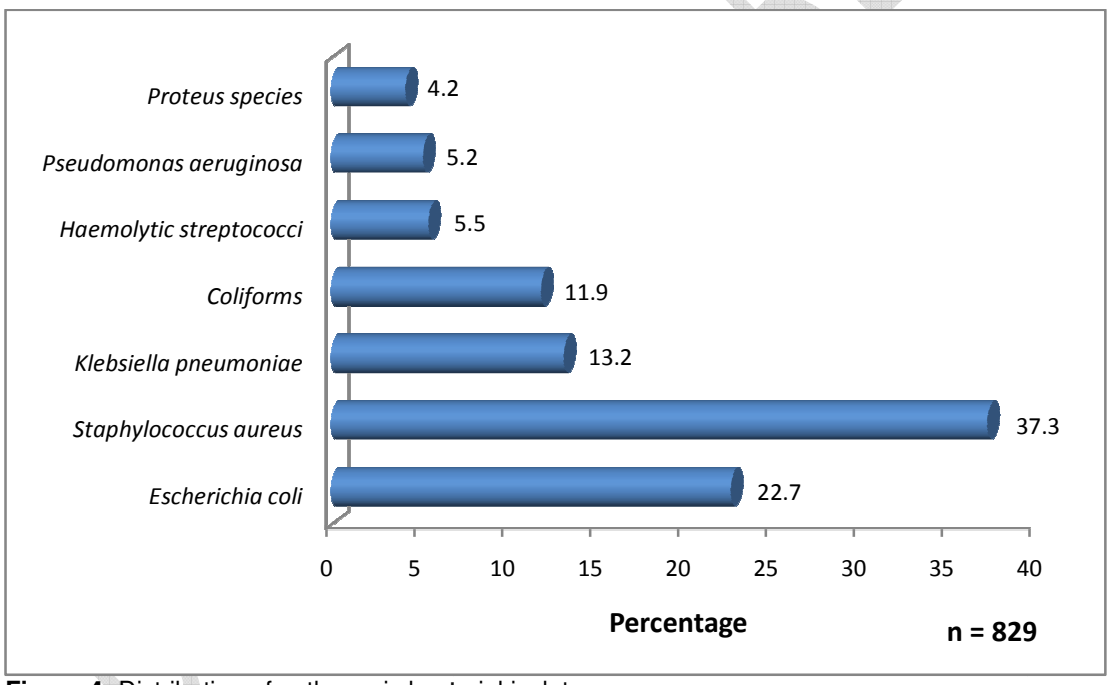

Figure 4: Distribution of pathogenic bacterial isolates

All the pathogenic isolates were resistant to between 3 – 6 commonly prescribed antibiotics in the hospital with an average resistance of 4 antibiotics [Table 2]

Table 2: Mean number of resistant antibiotic strains [n = 829]

Bacteria	Number	Antibiotic resistant strains Mean ± SD
Escherichia coli	188	4.06 ± 1.78
Staphylococcus aureus	309	4.11 ± 1.92
Klebsiella pneumonia	109	4.82 ± 1.57
Coliforms	99	4.18 ± 1.72
Proteus spp	35	4.56 ± 1.18
Pseudomonas aeruginosa	43	4.58 ± 1.38
Haemolytic streptococci	46	3.78 ± 1.42

The prevalence of multidrug resistant strains showed that resistance was found in about 90% of *Staphylococcus aureus* and over 50% of *Escherichia coli* isolates. A similar pattern of resistance was observed for other bacterial isolates though resistance level was lower [Figure 5]

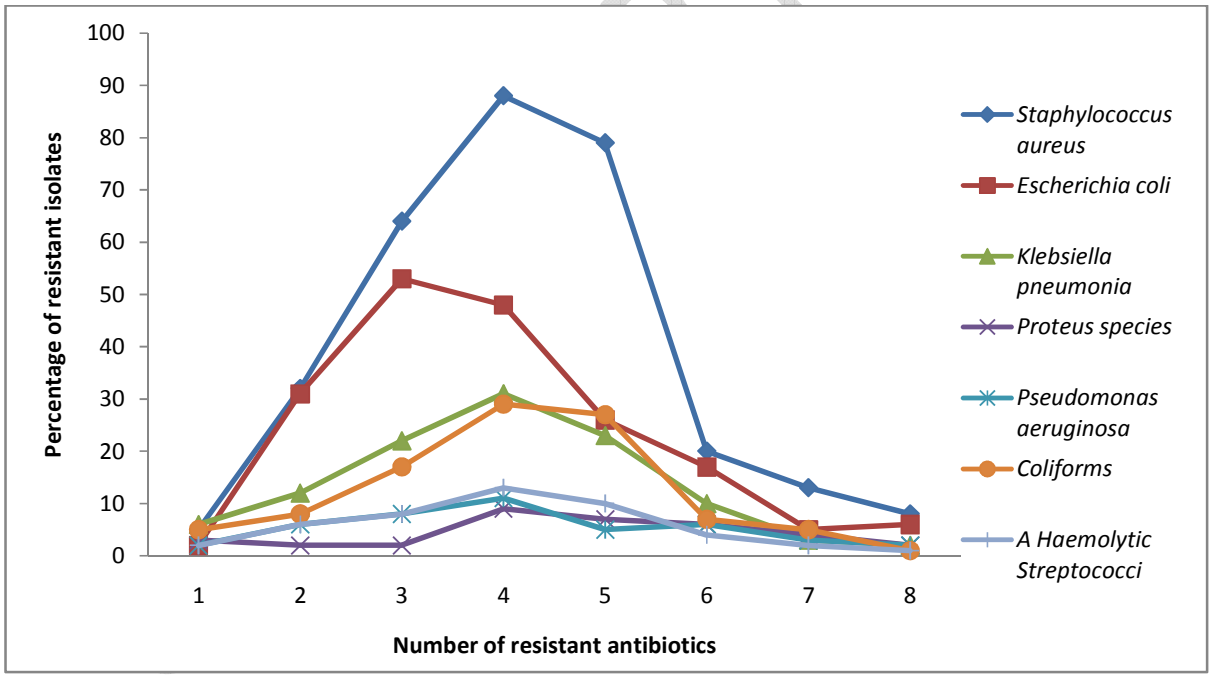

Figure 5: Percentage of multidrug resistant isolates

The pattern of resistance showed that it was highest with the Penicillins and Cotrimoxazole, moderate with Ceftriaxone, Tetracycline, Erythromycin and Gentamycin and least with the Quinolones [Table 3]

Table 3: Resistance to antibiotics

Drug	EC[%] n = 188	SA[%] n = 309	COL[%] n = 99	KP[%] n = 109	PT[%] n = 35	PS[%] n = 43	HS[%] n = 46	Resistance range [%]
Cloxacillin	30[15.9]	154[49.3]	22[22.2]	29[26.6]	4[11.4]	6[13.9]	17[36.9]	11.4 – 49.8
Clindamycin	21[11.2]	36[11.5]	17[17.2]	21[19.3]	1[2.1]	7[16.3]	17[36.9]	2.1 – 36.9
Amx + CLA	131[69.7]	208[66.7]	86[86.9]	94[86.2]	23[65.7]	20[46.5]	15[32.6]	32.6 – 86.9
Cotrimoxazole	110[58.5]	183[58.7]	67[67.7]	81[74.3]	18[47.4]	15[34.9]	18[39.1]	34.9 – 74.3
Clarithromycin	19[10.1]	74[23.7]	18[18.2]	22[20.2]	6[17.1]	7[16.3]	4[8.7]	8.7 – 23.9
Tetracycline	60[31.9]	56[17.9]	38[38.4]	51[46.8]	17[48.6]	9[20.9]	5[10.8]	10.8 – 48.6
Ceftriaxone	51[27.1]	46[14.7]	31[31.3]	50[45.9]	6[17.1]	7[16.3]	2[4.3]	4.3 – 45.9
Gentamycin	36[19.1]	33[10.6]	28[28.3]	41[37.6]	9[23.7]	2[4.7]	NA	4 7 – 37.6
Methicillin	11[5.9]	8[2.6]	10[10.1]	9[8.2]	5[14.3]	NA	NA	2.6 – 14.3
Erythromycin	12[6.4]	108[34.6]	14[4.1]	21[19.3]	3[3.6]	7[16.3]	10[21.7]	3.6 – 34.9
Ofloxacin	19[10.1]	10[3.2]	13[13.1]	14[12.8]	3[8.6]	NA	NA	3.2 – 13.1
Levofloxacin	7[3.7]	8[2.6]	5[5.1]	10[9.2]	NA	NA	NA	2.6 – 9.2
Ciprofloxacin	4[2.1]	23[7.4]	5[5.1]	9[8.2]	1[2.9]	1[2.3]	9[19.6]	2.1 – 19.6
Nalidixic acid	9[4.8]	18[5.8]	9[9.1]	12[11.0]	3[8.6]	1[2.3]	2[4.3]	2.3 – 11.0
Ampiclox	NA	15[4.8]	2[2.0]	1[0.9]	1[2.9]	NA	9[19.6]	1.0 – 19.6
Amoxicillin	1[0.5]	20[6.4]	5[5.1]	3[2.7]	4[11.4]	1[2.3]	14[30.4]	1.0 – 30.4
Norbactin	2[1.1]	20[6.4]	4[4.0]	6[5.5]	2[5.7]	1[2.3]	10[21.7]	1.1 – 21.7
Perfloxacin	1[0.5]	8[2.6]	3[3.0]	5[4.6]	1[2.9]	NA	NA	1.0 – 4.6
Streptomycin	1[0.5]	13[4.2]	3[3.0]	3[2.7]	1[2.9]	NA	NA	1.0 – 4.2

***Key**: EC = Escherichia coli, SA = Staphylococcus aureus, COL = Coliforms, KP = Klebsiella pneumonia, PT = Proteus spp, PS = Pseudomonas aeruginosa, HS = Haemolytic streptococci, AMX+CLA = Amoxicillin + Clavulanic acid, NA = not applicable*

Discussion: The emergence and rapid spread of resistance in sub Saharan Africa is endangering efficacy of antibiotics and limiting treatment options in the face of high infectious disease burden. Healthcare facilities have been recognized as a place where resistance to antibiotics can easily be spread among patients. The results of this study showed that *Staphylococcus aureus* accounted for more than a third of all isolates from clinical samples followed by *Escherichia coli*. These two bacteria account for more than two thirds of all isolates which is comparable to earlier study [Masyeni *et al*, 2018], but lower than that reported in several previous studies [Sewunet *et al*, 2013, Dilnessa *et al*, 2016]. Many clinical samples identified *Staphylococcus aureus,* however *Escherichia coli* were predominantly found in urine samples [Ragbetli *et al*, 2016, Ramirez – Castillo *et al*, 2018]. Several studies reported that *Staphylococcus aureus* is found in many clinical specimens across African countries with prevalence that is as high as 60.9% [Acquah *et al*, 2013, Opoku-Okrah *et al*, 2013]. A number of

gram negative bacteria such as *Klebsiella, Proteus* and *Pseudomonas aeruginosa* have been reported clinical specimens with varying level of prevalence [Mordi *et al*, 2009, Kehinde *et al*, 2004, Fadeyi *et al*, 2016]. Majority of alpha *haemolytic Streptococci* were isolated from sputum specimens similar to previous studies [Masyeni *et al*, 2018].

In many developing countries, prevalence of *Klebsiella* infections is higher compared to the findings of this study [Hansen *et al*, 2004, Chakraborty *et al*, 2016, Olowe *et al*, 2012]. Similar pattern of varying prevalence of bacterial isolates was reported for *Pseudomonas aeruginosa*, *Proteus* species ,*Klebsiella* and *coliforms* which are in contrast to the results of this study [Mahmoud et al 2016, Patil *et al*, 2017, Akter *et al*, 2014, Raiz *et al*, 2012, Sarathbau *et al*, 2012, Prasad *et al,* 2016, Bahashwan *et al*, 2013]

The emergence of antibiotic resistance is known to be due to a complex interplay of several factors that include overuse/irrational use and the environmental factors. Evidence from this study showed that bacterial isolates were resistant to 3 – 6 antibiotics on the average. This high level of resistance presents a unique challenge in this setting where empirical antibiotic treatment is widespread. It also raises doubt as to the efficacy and appropriateness of existing guideline recommendations for syndromic treatment of infections {Bernabe *et al*, 2017]. Antibiotics with high level resistance included Amoxicillin + Clavulanic acid, Cotrimoxazole, Cloxacillin, Tetracycline and Ceftriaxone in that order of decreasing frequency. Quinolones have the least resistance which were below 20% for these commonly isolated bacteria.

The level of resistance to Amoxicillin + Clavulanic acid in this study is lower than that earlier reported [Ragbetli *et al*, 2016, Saba *et al*, 2017, Bernabe *et al*, 2017], but comparable to results of other studies [Masyeni *et al*, 2016]. Resistance of *Staphylococcus aureus* to Cotrimoxazole and Macrolides in this study is considerably higher compared to some previous studies [Aydin *et al*, 2001, Ozkalp *et al*, 2003]. In the case of *Escherichia coli*, resistance to Amoxicillin + Clavulanic acid, Cotrimoxazole and Ceftriaxone, it is comparatively higher [Ray *et al*, 2015, Ali Abdel Rahim *et al*, 2014]. Penicillins and Macrolides have showed consistently comparable level of resistance to several strains of bacteria as in previous studies [Dash *et al*, 2013, Niranjan *et al*, 2014, Dugal *et al*, 2013]. The level of resistance to Quinolones found in this study is lower than previous studies, though some reports indicated that bacterial resistance is high [Olorunmola *et al*, 2013, Akter *et al*, 2014].

A similar pattern of resistance was also observed with *Pseudomonas aeruginosa* and *Haemolytic Streptococci*, though their resistance to Quinolones have been reported to have higher [Sharma *et al*, 2016, Khan *et al*, 2014], even though some studies reported lower level of resistance [Naik *et al*, 2016]. The high level of multidrug resistance observed in this study has also been reported around the world [Rossolini *et al*, 2014, Golkar *et al*, 2014]. One of the major driving factors is inappropriate prescribing and self-medication in the community [Bartlett *et al*, 2013, Luyt *et al*, 2014]. In Nigeria, poor regulatory controls and inappropriate prescription of antibiotics is compounding the problems of resistance as many patients only report to hospital when self-medication fail to address their health problems.

Evidence from this study and several others clearly indicate that routine empirical antibiotic prescription can no longer be justified as rational. There is therefore an urgent need to review antibiotic use policies to emphasize microbial susceptibility testing as a means of ensuring that cost effective treatment outcomes are achieved.

Conclusion: Antibiotic resistance to commonly used antibiotics is very high. There is need to de-emphasize empirical prescriptions and give way for evidence based susceptibility testing of pathogens before initiation of a suitable course of antibiotic therapy.

Limitations: There are a number of limitations of this study and they include.

- The data were extracted from records and there may be errors in entry and/or test procedures
- The quality of materials and adherence to standard test procedures could not be ascertained
- The presence of antibiotic tainted samples due to previous antibiotic therapy or self-medication may influence results

Conflict of interest: The authors declare that there is no conflict of interest

References

1. Acquah SE, Quaye L, Sagbe K, Ziem JB, Bromberger P, Amponsem AA. Susceptibility of bacterial etiological agents to community used antimicrobial agents in Children with sepsis at the Tamale teaching hospital. BMC Infect Dis 2013. 18(13): 89
2. Adjei O, Opoku C. Urinary tract infections in African infants. Int J Antimicrob Agents 2004. 24(Suppl 1): S32 – S34
3. Akinyandenu O, Akinyandenu A. Irrational use and non-prescription sale of antibiotics in Nigeria: A need for change. J Sci Innovative Res 2014. 3(2): 251 – 257
4. Akram A, Megha R, Irfanul H, Pravina A, Rahul I, Dasari R, Kuriakose S, Patel I. Study the prescription pattern of antibiotics in the medicine department in a teaching hospital: A descriptive study. Int J Toxicol Pharmacol Res 2014. 6(4): 43 – 46
5. Akter J, Masadil Azad Chowdhury AM, Forkan M. Study on prevalence and antibiotic resistance pattern of *Klebsiella* isolated from clinical samples in South east region of Bangladesh. Am J Drug Delivery Dev 2014. 4(1): 73 – 79
6. Ali Abdel Rahim KA, Ali Mohamed AH. Prevalence of extended spectrum beta – lactamase producing *Klebsiella pneumoniae* in clinical isolates. Jundishapur J Microbiol 2014. 7(11): e17114
7. Amabile – Cuevas CF, Arredondo – Garcia JL, Cruz A, Rosas I. Fluoroquinolone resistance in clinical and environmental isolates of *Escherichia coli* in Mexico City. J Appl Microbiol 2010. 168(1): 158 – 162
8. Aydin N, Gultekin B, Eyigor M, Gurel M. The antibiotic resistance of Staphylococuss aureus isolated of clinical specimens. Adan Menderes Universitesi Tip Fakultesi Dergisi 2001. 2(3): 21 – 26
9. Bahashwan SA, El Shafey HM. Antimicrobial resistance pattern of *Proteus* isolates from clinical isolates from clinical specimens. Europ Sci J 2013. 9(27): 1857 – 1881
10. Bartlett JG, Gilbert DN, Spellberg B. Seven ways to preserve the miracle of antibiotics. Clin Infect Dis 2013. 56(10): 1445 – 1450
11. Bernabe KJ, Langendorf C, Ford N, Baptiste Ronat J, Murphy R. Antimicrobial resistance in West Africa: a systematic review and meta – analysis. Int J Antimicrob Agents 2017. 50: 629 – 639
12. Blomberg B, Jureen R, Manji RP, Tamin BS, Nwakagile DSM, Urassa WK et al. High rate of fatal causes of pediatric septicemia caused by gram negative bacteria with extended spectrum beta-lactamases in Dar es Salam, Tanzania. J Clin Microbiol 2005. 43: 745 – 749
13. Bubonja-Sonje M, MatovinaM, Skrobonja J, Bedemic B, Abram M. Mechanism of Carbanepem resistance in multidrug resistant clinical isolates of *Pseudomonas aeruginosa* from a Croatian hospital. Microbiol Drug Resist 2015. 21(3): 261 – 269
14. Center for disease dynamics, economics and policy 2015. State of the world's antibiotics 2015. Washington DC.

15. Chakraborty S, Moshina K, Sarker PK, Alam MDZ, Sayem SMA. Prevalence, antibiotics susceptibility profiles and ESBL production in *Klebsiella pneumoniae* and *Klebsiella oxytoca* among hospitalized patients. Periodicum Biologocum 2016. 118(1): 53 – 58
16. Cui D, Liu X, Hawkey P, Li H, Wang Q, Mao Z, Sun J. Use of and microbial resistance to antibiotics in China: a path to reducing antimicrobial resistance. J Int Med Res 2017. 45(6): 1768 – 1778
17. Dash M, Padhi S, Mohanty I, Panda P, Parida B. Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India. Indian J Family Community Med 2013. 20: 20 – 26
18. Dehbanipour R, Rastaghi S, Sedighi M, Maleki N, Faghri J. High prevalence of multidrug resistance uropathogenic *Escherichia coli* strain. Isfahan, Iran. J Nat Sci Biol Med 2016. 7(1): 22 – 26
19. Dilnessa T, Bitwe A. Prevalence and antimicrobial susceptibility pattern of Methicillin resistance *Staphylococcus aureus* isolated from clinical samples at Yekatit 12 hospital medical college, Adis Ababa, Ethiopia. BMC Infect Dis 2016: 398
20. Dugal S, Purohit H. Antimicrobial susceptibility profile and detection of extended spectrum beta-lactamases production by gram negative uropathogens. Int J Pharm Pharm Sci 2013. 5: 434 – 438
21. Eshiet UI, Effiong GS, Akwaowoh AE. The use of antibiotics in a Nigerian tertiary health care facility. Am J Biomed Sci Engineering 2015. 1(5): 25 – 31
22. Frean J, Perovic O, Fensham V, McCarthy K, von Gottberg A, de Gouveia L et al. External quality assessment of national public health laboratories in Africa 2002 – 2009. Bull World Health Organization 2012. 90: 191 – 191A
23. Golkar Z, Bagazra O, Pace DG. Bacteriophage: a potential solution for the antibiotic resistance crisis. J Infect Dev Ctries 2014. 13. 8(2): 129 – 136
24. Gordon JR, Lowy FD. Pathogenesis of MRSA infection. Clin Infect Dis 2008. 46(5): S350 – S359
25. Hansen DS, Auken HM, Abiola T, Podschun R. Recommended test panel for differentiation of *Klebsiella* species on the basis of trilateral inter-laboratory evaluation of 18 biochemical tests. J Clin Microbiol 2004. 42: 3665 – 3669
26. Haznedaroglu T, Oncul T, Hosbul S, Cavuslu O, Ozyurt M et al. Methicillin resistance in *Staphylococcus aureus* strains isolated from hospitalized patients: three year trend. TAF Prevent Med Bull 2010. 9(6): 585 – 590
27. *Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobacteriacea: mechanism and clinical implications. Br Med J 2016. 352. Article ID h6420*
28. Jarvis WR, Schlosser J, Chinn RY, Tweeten S, Jackson M. National prevalence of Methicillin resistant *Staphylococcus aureus* in inpatients at a US healthcare facilities. Am J Infect Control 2007. 35(10): 631 – 637
29. Jombo GT, Emanghe UE, Amefule EN, Damen JG. Urinary tract infections at a Nigerian University hospital, causes and antimicrobial susceptibility profile. J Microbiol Antimicrob 2011. 3: 53 – 59
30. Kariuki S, Gordon MA, Feasey N, Parry CM. Antimicrobial resistance and management of invasive Salmonella disease. Vaccine 2015. 33(Suppl 3): C21 – C29
31. Kaur A, Bhagat R, Kaur N, Shafiq N, Gautam V, Malhotra S, Suri V, Bhalla A. A study of antibiotic prescription pattern in patients referred to tertiary care center in Northern India. Ther Adv Infect Dis 2018. 5(4): 63 – 68

298 32. Kehinde AO, Ademola SA, Okesola AO, Oluwatosin OM, Bakare RA. Pattern of bacterial pathogens in burn
299 wound infection in Ibadan, Nigeria. Ann Burns fire Disasters 2004. 17(1): 12 – 15
300 33. Kesah C, Ben Redjeb S, Odugbemi TO, Boye CS et al. Prevalence of Methicillin resistant *Staphylococcus*
301 *aureus* in eight African countries and Malta. Clini Microbiol Infect 2003. 9: 153 – 156
302 34. Khan F, Khan A, Kazmi SU. Prevalence and susceptibility pattern of multidrug resistant clinical isolates of
303 *Pseudomonas aeruginosa* in Karachi. Pak J Med Sci 2014. 30(5): 951 – 954
304 35. Kourlaba G, Gkrania-Klotsas E, Kourkouni E, Mavrogeorgos G, Zaoutis TE. Antibiotic prescribing and
305 expenditures in outpatient adults in Greece, 2010 – 2013: Evidence from real world practice. Euro Surveill
306 2016. 21(26): PII – 302266.
307 36. Labarca JR, Salles MJ, Seas C, Guzman-Blanco M, Carbanepem resistance in Pseudomonas aeruginosa
308 and Acinebacter baumanii in the nosocomial setting in Latin America. Critical Rev Microbiol 2016. 42(2):
309 276 – 292
310 37. Lateef A. Antibiotics use and misused in developing countries. World J Microbiol 2004. 20: 167 – 170
311 38. Le Doare K, Bielicki J, Heath PT, Sharland M. Systematic review of antibiotic resistance rates among gram
312 negative bacteria in children with sepsis in resource limited countries. J Pediatr Infect Dis Soc 2014. 4(1):
313 11 – 20
314 39. Leopold SJ, van Leth F, Tarekegn H, Schultsz C. Antimicrobial drug resistance among clinically relevant
315 bacterial isolates in sub Saharan Africa: A systematic review. J Antimicrob Chemother 2014. 69(9): 2337 –
316 2353
317 40. Levy SR, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med
318 2004. 10(Suppl 12): S122 – S129
319 41. Lim MK, Lai PS, Ponnampalavanar SS, Syed Omar SF, Taib NA, Yusof MY, Italiano CM, Kong DC,
320 Kamarulzaman A. Antibiotics in surgical wards: Use or misuse: A newly industrialized country's perspective.
321 J Infect Dev Ctries 2015. 9: 1264 – 1271
322 42. Luyt CE, Brechot N, Trouillet JL, Chastre J. Antibiotic stewardship in the intensive care unit. Crit Care 2014.
323 18(5): 480
324 43. Mahmoud AM, Tarig MSA, Osama MS, Mariam MA. Prevalence and antimicrobial resistance pattern of
325 bacterial strains isolated from patients with urinary tract infections in Messalata central hospital, Libya.
326 Asian Pacific J Trop Med 2016. 9(8): 771 – 778
327 44. Masyeni S, Sukmawati H, Siskayani AS, Dharmayanti S, Sari K. Antimicrobial susceptibility pattern of
328 pathogens isolated from various specimens in Denpasar – Bali: A two years retrospective survey. Biomed
329 Pharmacol J 2018. 11(1): 493 – 502
330 45. Mava Y, Bello M, Ambe JP, Zailani SB. Antimicrobial sensitivity pattern of organisms causing urinary tract
331 infections in children with sickle cell anaemia in Maiduguri, Nigeria. Niger J Clin Pract 2012. 15: 420 – 423
332 46. Misic AM, Gardner SE, Grice EA. The wound microbiome: modern approaches to examining the role of
333 microorganisms in impaired chronic wound healing. Adv Wound Care 2014. 3(7): 502 – 510
334 47. Mordi RM, Momoh MI. Incidence of Proteus species in wound infection and their sensitivity pattern in
335 University of Benin teaching hospital. Afr J Biotech 2009. 8(5): 725 – 730

48. Muluye D, Wondimeneh Y, Ferede G et al. Bacterial isolates and their antibiotic susceptibility pattern among patients with pus and /or wound discharge at Gondar University hospital. BMC Res Notes 2014. 7(1): 619
49. Muoneke V, Ibekwe M, Ibekwe R. Childhood urinary tract infections in Abakiliki: etiological organisms and antibiotic sensitivity pattern. Ann Med Health Sci Res 2012. 2: 29 – 32
50. Naik TB, Nadagir SD, Biradar A. Prevalence of Beta – *Hemolytic Streptococci* groups A, C and G in patients with acute pharyngitis. J Lab Physician 2016. 8(1): 45 – 49
51. Niranjan V, Malini A. Antimicrobial resistance pattern in a *Escherichia coli* causing urinary tract infections among inpatients. Indian J Med Res 2014. 139: 945 – 948
52. Nsofor CA, Anyanwu NC, Ogbulie TE. High antibiotic resistance pattern observed in bacterial isolates from a tertiary hospital in South east Nigeria. Int J Res Pharm Biosciences 2016. 3(1): 1 – 6
53. Nsofor CA, Iroegbu CU. Antibiotic resistance profile of Escherichia coli isolated from apparently healthy livestock in south eastern Nigeria. J Cell Animal Biol 2012. 6(6): 2445 – 2450
54. Oduyebo OO, Olayinka AT, Iregbu KC, Versporten A, Goossens H, Nwajiobi-Princewill PI, Jimoh O, Ige TO, Aigbe AI, Ola-Bello OI, Aboderin AO, Ogunsola FT. A point prevalence survey of antimicrobial prescribing in four Nigerian tertiary hospitals. Ann Trop Pathol 2017. 8: 42 – 46
55. Ohi CA, Luther VP. Antimicrobial stewardship for inpatients facilities. J Hosp Med 2011. 6(Suppl 1): S4 – S15
56. Okwori EE, Nwadioha SI, Jombo GTA, Nwokedi EOP, Odimayo MS. A comparative study of bacterial isolates from the urine samples of AIDS and non AIDS patients in Benue, Nigeria. Asian Pacific J Trop Med 2010. 3: 382 – 385
57. Oladeinde BH, Omoregie B, Olley M, Anunibe JA. Urinary tract infection in a rural community of Nigeria. N Am J Med Sci 2011. 3: 75 – 77
58. Oli AN, Okafor CI, Ibezim EC, Akujiobi CN, Onwunzo MC. The prevalence and bacteriology of asymptomatic bacteruria among antenatal patients in Nnamdi Azikiwe University teaching hospital Nnewi, South eastern Nigeria. Niger J Clin Pract 2010. 13: 409 – 412
59. Olorunmola FO, Kolawole DO, Lamikanra A. Antibiotics resistance and virulence properties in *Escherichia coli* strains from cases of urinary tract infections. Afr J Infect Dis 2013. 7(1): 1 – 7
60. Olowe OS, Oladapo GO, Makajuola OA, Olaitan JO. Prevalence of extended spectrum beta – lactamases [ESBLs] carrying genes in *Klebsiella* species from clinical samples at Ile – Ife, South west Nigeria. Int J Pharm Med Biosci 2012. 1(2): 129 – 138
61. Opoku – Okrah C, Feglo P, Amidu N, Dekorah MP. Bacterial contamination of donor blood at the Tamale teaching hospital, Ghana. Afr Health Sci 2009. 9: 13 – 18
62. Ozkalp B, Baybek H. Invitro susceptibility to various antibiotics of *Staphylococcus aureus* isolated from clinical specimens. Genel Tip Derg 2003. 13(2): 65 – 68
63. Paniagua-Contreras GL, Monroy-Perez E, Rodriguez-Moctezuma JR, Dominguez-Trejo P, Vaca-Paniagua F, Vaca S. Virulence factors, antibiotic resistance phenotypes and O sero-groups of *Escherichia coli* strains isolated from community acquired urinary tract infection patients in Mexico. J Microbiol Immunol Infect 2017. 50(4): 478 – 485

64. Pitout JD, Laupland KB. Extended spectrum beta-lactamase producing Enterobacteriacea: an emerging public health concern. Lancet Infect Dis 2008. 3(3): 159 – 166
65. Prasad RR, Shree V, Sagar S, Kumar S, Kumar P. Prevalence and antimicrobial susceptibility pattern of *Proteus* species in clinical samples. Int J Curr Microbiol App Sci 2016. 5(4): 962 – 968
66. Putil HV, Patil VC. Incidence, bacteriology and clinical outcome of ventilator associated pneumonia at tertiary care hospital. J Natr Sci Biol Med 2017. 8(1): 46 – 55
67. Rabasa AI, Shattima D. Urinary tract infections in severely malnourished children at the University of Maiduguri teaching hospital. J trop Pediatr 2002. 48: 359 – 361
68. Ragbetli C, Parlak M, Bayram M, Guducuoglu H, Ceylan N. Evaluation of antimicrobial resistance in *Staphylococcus aureus* isolates by years. Interdisciplinary Perspect Infect Dis 2016. ID9171395. http://dx.doi.org/10.1155/2016/9171395
69. Ragbetli C, Parlak M, Bayram Y, Guducuoglu H, Ceylan N. Evaluation of antimicrobial resistance in *Staphylococcus aureus* isolates by years. Interdisciplinary Perspect Infect Dis 2016. ID 9171395. http://dix.doi.org/10.1155/2016/9171395
70. Ramirez – Castillo FY, Moreno – Flores A, Avelar – Gonzalez FF, Marquez – Diaz F, Harel J, Guerrero – Barrera AL. An evaluation of multidrug resistant *Escherichia coli* isolates in urinary tract infections from Aquascalientes. Mexico: cross sectional study. Ann Clin Microbiol 2018. 17: 34
71. Ramirez-Castillo , Flor Y, Adriana C, Moreno-Flores, Francisco J, Avelar-Gonzalez, Francisco –Marquez-Diaz, Josee H, Alma L, Guerrero-Barrera. An evaluation of multidrug resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: Cross section study. Ann CLin Microbiol Antimicrob. 2018. 17: 34
72. Riaz S, Faisal M, Hasnain S. Prevalence and comparism of beta-lactamase producing *Escherichia coli* and *Klebsiella* species from clinical and environmental sources in Lahore, Pakistan. Afr J Microbiol Res 2012. 6: 465 – 470
73. Rice LB. Antimicrobial resistance in gram positive bacteria. Am J Med 2006. 119(6): Suppl 1. S11 – S19
74. Robicsek A, Beaumont JL, Paule SM et al. Universal surveillance for Methicillin resistant *Staphylococcus aureus* in 3 affiliated hospitals. Ann Intern Med 2008. 148(6): 409 – 418
75. Rossolini GM, Arena F, Pecile P, Pollini S. Update on the antibiotic crisis/ Clin Opin Pharmacol 2014. 18: 56 – 60
76. Ruiz J, Villareal E, Gordon M, Frasquet J, Castellanos A, Ramirez P et al. From MIC creep to MIC decline : *Staphylococcus aureus* antibiotic susceptibility evolution over the last 4 years. Clin Microbiol Infect 2016. 22(8): 741 – 742
77. Russo TA, Johnson JR. Medical and economic impact of extra-intestinal infections due to *Escherichia coli*: focus on an increasing important endemic problem. Microbes Infect 2003. 5(5): 449 -456
78. Sarathbau R, Ramani TV, Bhaskara K, Panda S. Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from sputum, urine and pus samples. J Pharm Biol Sci 2012. 1: 4 – 9
79. Sewunat T, Demissie Y, Mihret A, Abebe T. Bacterial profile and antimicrobial susceptibility pattern of isolates among burn patients at Yekatit 12 hospital burn centre, Adis Ababa, Ethiopia. Ethiopian J Health Sci 2013. 23(3): 209 – 216

80. Sharma J, Singh S, Kaur Gill A, Kaur A. Prevalence and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolated from pus samples in a tertiary care hospital, Bathinda. Int J Contemporary Med Res 2016. 3(12): 3481 – 3483
81. Silva-Sanchez J, Cruz-Trujillo E, BarriosH, Reyna-Flores F, Sanchez-Perez A, Garcia-Ramos U. Characterization of plasmid mediated Quinolone resistance [PMQR] gene in extended spectrum beta-lactamase producing Enterobacteriacea in pediatric clinical isolates in Mexico. PlosOne 2013. 8(10): e77968
82. Sindhulina C, Geethalakshmi S, Thenmozhivalli PR, Jose JM, Brahmadathan KN. Bacteriological and molecular studies of group A Streptococcal pharyngitis in a south Indian hospital. Indian J Med Microbiol 2008. 26: 197 – 198
83. Sire JM, Nabeth P, Perrier-Claude JD, Bahsoun I, Silby T, Macondo EA et al. Antimicrobial resistance in outpatient *Escherichia coli* urinary isolates in Dakar – Senegal. J Infect Dev Ctries 2007. 1: 263 – 268
84. Stamm WE, Norby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis 2001. 183(Suppl 1): S1 – S4
85. Trojan R, Razdan L, Singh N. Antibiotic susceptibility pattern of bacterial isolates from pus samples in a tertiary care hospital of Punjab, India. Int J Microbiol 2016. Article ID 9302692. *http://dx.doi.org/10.1155/2016/9302692*
86. Umar LH, Isah A, Musa S, Umar B. Prescribing pattern and antibiotic use for hospitalized children in a northern Nigerian teaching hospital. Ann Afr Med 2018. 17: 26 – 32
87. Urban C, Mariano N, Bradford PA, Tuckman M, Segal-Maurers JR et al. Identification of CTX-M- beta-lactamases in *Escherichia coli* from hospitalized patients and residents of long term care facilities. Diagn Microbiol Infect Dis 2010. 66(4): 402 – 406
88. World Health Organization. Antimicrobial resistance – global report on surveillance. Geneva. Switzerland. WHO 2014
89. World Health Organization. The evolving threat of antimicrobial resistance: Options for action. Geneva, Switzerland. WHO 2012
90. Zimmer BL. Combating antibiotic resistant bacteria. The microbiology laboratory answers the challenge. MLO Med Lab Obs 2015. 47: 30 – 33