Original article

Antibiotics Susceptibility Patterns of Bacteria Isolated from Clinical Samples in Thamar, Yemen

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Abstract

This study was designed to determine the antimicrobial sensitivity patterns of bacterial isolates from patients who visited different hospitals and medical laboratories in Thamar City, Yemen. 135 specimens collected from various clinical samples: blood (62), urine (38), stool (35) were analyzed and the organisms were identified using colonial morphology, gram staining, appropriate biochemical test and API20E. The identified organisms include *Salmonella typhi*, *S. paratyphi, Escherichia coli, Klebsiella pneumonia*, *Shigellae sp Salmonella sp., Citrbacteir freundi*, *Enterobacter sp.* Antibiotics susceptibility studies showed that *S. typhi* isolates were highly resistant to lincomycin, tetracycline and most were resistant to rifampicin and nalidixic acid. Intermediate resistance was observed to ciprofloxacin and Norfloxacin and all were susceptible to amoxicillin, doxycycline, streptomycin, gentamycin and ampicillin. *S. paratyphi* isolates showed resistance to lincomycin, tetracycline, streptomycin and nalidixic acid but susceptible to amoxicillin, doxycycline and gentamycin. *E. coli* isolates showed high resistance to ampicillin, erythromycin, lincomycin, amoxicillin and rifampicin and ciprofloxacin, tetracycline, doxycycline, streptomycin, gentamycin and nalidixic acid. *K. pneumoniae* isolates showed high resistance to erythromycin, lincomycin, rifampicin, streptomycin, amoxicillin and ciprofloxacin but moderate resistance to tetracycline, nalidixic acid and doxycycline. *Shigellae species* isolates were resistant to erythromycin, lincomycin, rifampicin and gentamycin and tetracycline, ampicillin, amoxicillin but less resistant to doxycycline. Most of *Shigellae species* isolates were susceptible to norfloxacin and ciprofloxacin. Non-typhoid *Salmonella species* isolates were multi-drug resistant to the antibiotics tested. Drug resistance was encountered in most of studied isolates was attributed to their prevailing usage and abuse.

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Introduction

Bloodstream, urine and stool infections are the most common causes of many diseases. These diseases were caused by opportunistic bacterial species, (e.g., *Staphylococcus aureus*, Escherichia coli, *Acinetobacter* spp, *Klebseilla pneumonia*), and fungi species (Wisplinghoff *et al*., 2004; Risan, 2016). Gram negative bacteria play important role in infectious diseases, but the *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae* and *Proteus mirabilis* now become the broad spectrum species involved in nosocomial infections (Anuj Nautiyal *et al*., 2015). Rapid diagnostic identification and characterization of pathogens are essential to guide therapy, to predict outcomes and to detect transmission events or treat failures (Hasman *et al*. 2014).

Antibiotics identification, action modes and bacterial resistance mechanisms have been researched topics in academia until recently in the pharmaceutical applications (Davies and Davies, 2010 metabolic pathways (Fisher and Mobashery, 2010).). Bacteria can be resistant one or more type of antibiotics, the mechanisms by which bacteria resist antibiotics include, alter the receptor for the antibiotic, decrease antibiotic amount that reaches the receptor by preventing entry or removal, antibiotic alteration or destroying and develop resistant. Resistance to commonly-prescribed antibiotics is an expanding global problem and has been observed in both developed and developing countries (Finch, 1998, Farrar, 1985, Rahal *et al*., 1997 and Tenvor, Hughes, 1996). Resistance has emerged even to newer, more-potent antimicrobial agents (Parry, 1989). A number of epidemics have recently occurred caused by multiply resistant organisms. The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes (Courvalin and Weber, 2005; Chikere *et al.*, 2008). In Yemen Typhoid fever and bacterial infections of the urinary and gastrointestinal tract are common and represent a frequent cause of morbidity in outpatients as well as a frequent cause of nosocomial infections in many hospitals.

Most infections are treated on an empirical basis. Clinical experience has indicated the presence of numerous cases resistant to conventional chemotherapy. Microbial resistance rates to commonly prescribed antibiotics have increased recently (Power, 2004).

Updated knowledge of the prevailing causal bacteria and their susceptibility patterns is important for the proper selection and use of antimicrobial drugs and for the development of an appropriate prescribing policy. Furthermore, history has taught us that if we do not use antibiotics carefully, they will lose their efficacy. As a response to these challenges, the present study was designed to determine the antibiotic sensitivity pattern of bacteria isolated from hospitalized patients in Thamar city, Yemen.

MATERIALS AND METHODS

Sample collection

Blood Sample were collected aseptically from Patients visited of Thamar general hospital and Taiba hospital in Thamar city. A total of 62 blood samples were collected from patients who were suspected of having typhoid fever according to presumptive diagnosis by a medical practitioner. Stool and urine samples were collected aseptically from Patients visited of Thamar general hospital, Taiba hospital, Mussali hospital and Aljarfi, Aziz,Alfa, Bynon medical laboratories in Thamar city. A total of 35 stool sample and 38 urine sample were collected from patients who were suffering of diarrhea and urinary tract infection(UTI). All samples were collected in sterile disposable containers and analyzed at laboratory of Biotechnology and food technology Dept. Faculty of Agriculture and veterinary medicine ,Thamar University.

Sample preparation and enrichment

2 ml of Blood samples were inoculated onto tubes contained Fluid Thioglycolate medium (Himedia,India) and incubated for 48hr at 37ºC for enrichment of *Salmonella* ssp. Stool samples and urine samples were inoculated onto tubes contained peptone water and incubated for 24hr at 37ºC.

Isolation

The tubes were examined daily for evidence of bacteria growth, including turbidity. Subcultures were as follows: from each positive blood tube, First: a loop full was transferred to MacConkey agar , *Salmonella-Shigella* agar (S.S agar) and Xylose lysine desoxycholate Agar (Himedia, India), streaked, incubated for 24-48 hours at 37 ºC. Stool samples were cultured on McConkey agar and Salmonella-Shigella agar (S.S agar) and incubated for 48 hours at 37 ºC. Urine samples were cultured on blood agar, Salmonella-

Shigella agar(S.S agar)and McConkey agar and incubated for 48 hours at 37 ºC. The isolates were stained by Gram stained and examined by light microscope (Collee *e.al*., 1996).

Identification

124 Isolates from cases with significant bacteria were purified and identified as follows**:**

Biochemical tests

Important biochemical tests (Oxidase test, SIM test, Urease test, Methyl Red/ Voges-Proskauer test, Citrate utilization, TSI test, and Catalase test) were conducted according to (Collee *et.al*., 1996, Baron. and Finegold, 1990 and Forbes *et.al*. 2007).

Api20E system

Identification of isolates was confirmed by biochemical tests on API 20 strips (BioMerieux, France). This system is devised for the biochemical identification of Enterobacteriaceae and other gram negative bacilli. It consists of 20 microtubes containing dehydrated media (each microtube consist of a tube and cupule section). The Api 20E system was performed according to the manufacture instructions.

Antibiotic susceptibility testing

The most frequently isolates were then subjected to antibiotic sensitivity testing by the disc diffusion method on Mueller-Hinton agar HIMEDIA India) according to the National Committee for Clinical Laboratory Standards and Manual of Antimicrobial Susceptibility Testing guidelines (NCCLS, 2002; Cheesbrough, 2006; Coyle, 2005; Okonko *et al*., 2009a,b). Commercially available antimicrobial discs (HIMEDIA, India) were used in the study and included: Nalidixi acid (30 mcg), Doxycycline (30 mcg), Erythromycin (15 mcg), Sterptomycin (25 mcg), Tetracycline 30 mcg), Amoxicillin (30 mg), Ciprofloxacin (5 mcg), Gentamicin (10 mcg), Ampicillin (10mcg), Rifampicin (15 mcg), , Norfloxacin (10 mcg) and Lincomycin (2 mcg). Plates were incubated at 35 ºC. Zones of inhibition were interpreted as resistant or sensitive using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method as described by Cheesbrough (2006). Interpretation of results was done using the zone of inhibition sizes. Zones of inhibition of _ 18 mm were considered sensitive, 13-17 mm intermediate and < 13 mm resistant.

Results

Our findings showed that out of 135, only 54 patients had positive blood culture and 35 patients had positive urine culture. All isolates were identified by the standard biochemical tests and further confirmed by API20E (Figure 1.).

Figure (1): API 20E results for isolated bacteria *Salmonella typhi*.

S. typhi, *S. paratyphi*, and *Klebsiella pneumoniae* were the most common pathogens isolated from cases of typhoid fever with the percentage of 31(57.4%), 13(24.1%) and 7(13**%)** respectively, from the total number of blood samples (Table 1). Other pathogens were isolated in a relatively few number. The most common urinary pathogens isolated were *E. coIi*, and *Klebsiella pneumonia* with the percentage of 20(57.1%) and 13 (37.1%) respectively, from the total number of urine samples. *Escherichia coli*, *Shigella* species and *Salmonella species* were the most common pathogens isolated from cases of diarrhea in this study with the percentage of 14(40%), 11(31.4%) and 4(11.4?%), respectively, from the total number of stool samples (Table 1).

Escherchia coli ,*Salmonella typhi* and *Klebsiella pneumonia* were the most frequently isolated bacteria pathogen from clinical samples with rate 34(27.4%), 33(26.6%) and 22(17.7%) respectively, from the total number of clinical samples (Table 1).

Organism	Number of positive samples (%)			
	Blood (n=54)	Urine (n=35)	Stool (n=35)	Total(observed growth) (n=124)
Salmonella typhi	57.4	0.0	5.7	26.6
Salmonella paratyphi	24.1	0.0	0.0	10.5
Escherichia coli	0.0	57.1	40	27.4
Klebsiella pneumonia	13	37.1	5.7	17.7
Citrbacteir freundi	5.6	0.0	0.0	2.4
Enterobacter sp.	0.0	0.0	5.7	1.6
Shigellae sp.	0.0	0.0	31.4	8.9
Salmonella sp.	0.0	5.7	11.4	4.8

Table (1) organisms isolated from different clinical samples

Susceptibility of bacterial isolates (percentage of isolates showing antibiotic resistance) from patients to antibiotics is shown in Table (2). All *Salmonella typhi* isolates showed high resistance (100%) to lincomycin and tetracycline whereas 29(87.5%) and 17(50%) were resistance to, rifampicin and nalidixic acid, respectively. However, All 33 pathogenic isolates were susceptible (100%) to amoxicillin, doxycycline, streptomycin and gentamycin . Among 33 *Salmonella typhi* only 24(72.7%) and 21(62.5%) were Intermediate resistance to ciprofloxacin and Norfloxacin, respectively compared with 20(66.7%)
and 12(37.5%) were susceptible to ampicillin and nalidixic acid, respectively. As shown in Table (2), all *Salmonella paratyphi* isolates were resistance(100%) to lincomycin and tetracycline whereas 11(84.6%) and 10(76.9%) were resistance to streptomycin and nalidixic acid, respectively. All 13 *Salmonella paratyphi* isolates were susceptible to amoxicillin, doxycycline (100%) and gentamycin(84.6%) (Table 2). All *Escherchia coli* isolates showed high resistance (100%) to ampicillin, erythromycin, lincomycin, amoxicillin and ,rifampicin whereas 23 isolates (67.6%) were resistance to ciprofloxacin, tetracycline, doxycycline and streptomycin. However, 20(58.8%) and 17(50%) isolates were resistance to gentamycin and nalidixic acid, respectively. As indicated in table 2. all 22 *Klebsiella pneumoniae* isolates showed high resistance rate (100%) to erythromycin, lincomycin ,rifampicin and streptomycin and 16(72.7%) to amoxicillin and ciprofloxacin. Also 14(63.6%), 12(54.6%) and 11(50%) isolates were resistance to tetracycline, nalidixic acid and doxycycline, respectively. In the current study 11 *Shigellae species* isolates showed high resistance (100%) to erythromycin, lincomycin, rifampicin and gentamycin whereas 10(90.9%),9(81.8%), 8(72.7%) and 6(54.5%) isolates were resistance to tetracycline, ampicillin, amoxicillin and doxycycline, respectively. Among 11 *Shigellae species* isolates only 8(72.7%) and 7(63.6%) were susceptible to Norfloxacin and ciprofloxacin, respectively. All 6 nontyphoid

Salmonella species isolates showed resistance (100%) to erythromycin, lincomycin, amoxicillin and rifampicin whereas 4(66.7%) isolates were resistance to ampicillin, tetracycline, streptomycin and gentamycin .While, 3(50%) isolates were resistance to ciprofloxacin, nalidixic acid, doxycycline and Norfloxacin (Table 2.)

Table 2: Antibiotics sensitivity patterns for pathogen isolates from clinical samples.

Organism	Susceptibility	Number of positive samples (%)											
		AMP	E	L	AMC	CIP	TE	RIF	NA	DO	NX	S	GN
Salmonella typhi	Sensitive	66.7	12.1	0.0	100	27.3	0.0	0.0	37.5	100	25	100	100
	Intermediate	33.3	144	0.0	0.0	72.7	0.0	12.5	12.5	0.0	62.5	0.0	0.0
	Resistant	0.0	43.9	100	0.0	0.0	100	87.5	50	0.0	12.5	0.0	0.0
Salmonella paratyphi	Sensitive	71.4	0.0	0.0	100	15.4	0.0	0.0	23.1	100	30.8	0.0	84.6
	Intermediate	28.6	46.2	0.0	0.0	69.2	0.0	38.5	0.0	0.0	53.9	15.4	15.4
	Resistant	0.0	53.9	100	0.0	15.4	100	61.5	76.9	0.0	15.4	84.6	0.0
Escherichia coli	Sensitive	0.0	0.0	0.0	0.0	0.0	32.4	0.0	0.0	0.0	0.0	0.0	41.2
	Intermediate	0.0	0.0	0.0	0.0	32.4	0.0	0.0	50	32.4	67.6	32.4	0.0
	Resistant	100	100	100	100	67.6	67.7	100	50	67.6	32.4	67.6	58.8
Klebsiella pneumoniae	Sensitive	27.3	0.0	0.0	0.0	0.0	31.8	0.0	18.2	40.9	27.3	0.0	86.4
	Intermediate	36.4	0.0	0.0	27.3	27.3	4.6	0.0	27.3	9.1	72.7	0.0	0.0
	Resistant	36.4	100	100	72.7	72.7	63.6	100	54.6	50	0.0	100	13.6
Shigellae species	Sensitive	0.0	0.0	0.0	27.3	63.6	9.1	0.0	36.4	36.4	72.7	0.0	0.0
	Intermediate	18.2	0.0	0.0	0.0	36.4	0.0	0.0	27.3	9.1	72.7	72.7	0.0
	Resistant	81.8	100	100	72.7	0.0	90.9	100	36.4	54.5	0.0	72.7	100
Salmonella species	Sensitive	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0	33.3	33.3
	Intermediate	33.3	0.0	0.0	0.0	50	0.0	0.0	50	50	50	0.0	0.0
	Resistant	66.7	100	100	100	50	66.7	100	50	50	50	66.7	66.7

DISCUSSION

Antimicrobial chemotherapy has conferred huge benefits to human health as a variety of microorganisms that were elucidated to cause infectious diseases are controlled by the proper use of antibiotics. In the 20th century the discovery of antibiotic was viewed that all infectious disease will be conquered in the near future (Power,2004). However, in response to the development of antimicrobial agents, microorganisms, that have acquired resistance to drugs through a variety of mechanisms have emerged and continue to plague human beings. The primary factor responsible for the development and spread of bacterial resistance is the injudicious use of antimicrobial agents (Urassa *et al*., 1997).In Yemen, infectious diseases caused by drug resistant bacteria are one of the most important problems in daily clinical practices as observed in the present study. The data generally reflect the seriousness of the antimicrobial resistance among bacterial pathogens in Yemen. Our findings showed that out of 124 bacteria isolated, *E. coli* (34) and *Salmonella typhi* (33)) are the most frequently isolated organism, followed by *Klebsiella pneumoniae* (22), *Salmonella paratyphi* (13), *Shigellae species* (11) and *Salmonella species*(6). *Salmonella typhi* was the commonest Gram-negative organisms from blood while *E. coli* and *Klebsiella pneumoniae* were the most commonest bacteria from urine also *E. coli* and *Shigellae species* were the most commonest bacteria from stool. This finding is in line with the work of Iffat *et al*., 2011; he showed that *E. coli* was the most frequently isolated organism from urine sample.

All the isolates displayed variable resistance and sensitivity to the antibiotics tested as detailed as shown in table 2. Strains *Salmonella typhi* showed high resistance rate to lincomycin, tetracycline, rifampicin and nalidixic, ciprofloxacin and Norfloxacin but were susceptible to amoxicillin, doxycycline, streptomycin and gentamycin as shown in table 2. Our results showed that *Salmonella paratyphi isolates* were resistance (100%) to lincomycin and tetracycline whereas 11(84.6%) and 10(76.9%) were resistance to streptomycin and nalidixic acid but were susceptible to amoxicillin, doxycycline and gentamycin. A study conducted by Krishnan *et al* (2009*)* reported that among 359 isolates, the resistance was found against ampicillin (100%), ciprofloxacin (0.27%), nalidixic acid (100%), and erythromycin (17.82%). Notable results were found with gentamycin, since no resistance was found. Krishnan *et al* (2009) also observed that 70 and 30% of the isolates were *Salmonella enterica* sero var *typhi* and *paratyphi*, respectively among which highly sensitive to ampicillin (84%) this findings can be correlated with ours but not exactly. Our results also correspond and differ with other studies carried out by other researchers (Bhan *et al*., 2005, Lovely *et al*., 2012, Stella *et al.*, 2011 ad Wain *et al*., 1998). Our study showed a high ampicillin, erythromycin, lincomycin, amoxicillin, rifampicin, ciprofloxacin, tetracycline, doxycycline, streptomycin, nalidixic acid and gentamycin resistance especially among *E. coli and Klebsiella pneumoniae* except for *Klebsiella pneumoniae* that is susceptible to gentamycin. Similar and differ findings regarding drug resistance patterns of *Klebsiella pneumoniae* and *E. coli* have been reported by other researchers (Atif *et al*., 2000, Al-nasrawi and Abu almaali.2009 , Almaziny, 2014, Iroha *et al*., 2013, Eldaif *et al*.,2015, Patrícia *et al*.,2017). Moreover, this study indicates that both *Shigellae species* and non-typhoid *Salmonella species* organisms were resistance to the most antibiotics tested. On the average this shows that more than 50% of the organism is resistance to the antibiotics (Table 2.). This pattern is comparable to

other studies carried out in some other parts of the world (Godwin *et al*., 2006, Al-nasrawi and Abu almaali, 2009, Eldaif *et al*., 2015, Debas *et al*., 2011).The overall prevalence of resistance of microorganisms to antimicrobial agents was notably high in the current study, data reflecting the high level of antibiotic resistance in the country as a whole, when compared to other countries. The variation in the sensitivity pattern and high resistant rate to these commonly used drugs could be attributed to the prevailing usage and abuse, and the common attitude of over-the-counter purchase of the drugs in the areas under study. This further suggests a relationship between antibiotic usage and the level of drug resistance encountered in this study. Selection for drug resistance has been associated with an increased and inappropriate use of antibiotics (Ayliffe, 1970). There is an inordinate and irrational use of antimicrobial agents in Yemen and in other developing countries. Multiple factors have led to the prevalence of antibiotic resistance: 1) the wide use of antibiotics due to the high prevalence of infectious diseases, 2) a shortage of physicians,3) selective prescribing due to cost constraints and the pressure of pharmaceutical companies' promotional activities, 4) lack of laboratory support in rural areas, and 5) the difficulties in distributing information regarding antibiotic resistance. An important contributing factor is the deliberate self-administration of antibiotics by patients themselves when they are ill with diarrheal diseases (Kunin *et al*.,1987) .Our findings stress the need for distributing reliable information about antibiotic resistance and for ongoing drug-resistance surveillance. Knowledge of drug resistance in bacteria is indispensable for the proper selection of antimicrobial drugs. Yemen, a poor and underdeveloped country, allots a substantial amount of its health-expenditure allocation to the purchase of drugs, especially antibiotics. Resistance studies assist health authorities in the formulation of their own drug policies. They are also important for the general practitioner in a remote area who may not have access to microbiology laboratory back-up and hence must depend on the prevailing knowledge of antibiotic-resistant bacteria. Numerous health organizations world-wide donate large amounts of antibiotics to developing countries like the Yemen to treat diarrhea and other diseases at the community level. The composition of such donations should be based on current knowledge of the common local pathogenic bacteria and their susceptibility patterns.

Conclusions

- High rates of drug resistance were found in most of the tested isolates studied. In developing countries like Yemen, self-medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable to treat themselves.
- Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates.
- Determining the antimicrobial patterns of the disease causing organisms will enable health institutions to restrict the use of antimicrobials and take active measures in preventing the spread of drug resistance in hospitals.
- The insight into the antibiotic susceptibility of clinical isolates profile in any community is very imperative and desirable for effective management of the clinical conditions considering the relative differences in the pattern of susceptibility and resistance of so many pathogens to antibiotics from one locality to another.
- Therefore, it is important for hospitals to improve the processes of care known to impact infection rates.
- The judicious use of antibiotics by health workers and efforts to control procurement and use of antibiotics officially in all localities in Yemen will probably help to limit the increasing rates of multi-drug resistance in pathogens.

References

Almaziny M. A. (2014). Isolation, identification, and profile of antibiotic resistance of bacteria in childhood febrile neutropenia patients. *European Journal of Experimental Biology*, 4(2):1-6.

Al-nasrawi A. and Abu Almaali H.M. (2009). Antibiotic sensitivity patterns of uropathogens isolated from females with urinary symptoms in Karbala. *J. of Kerbala Uni.*, 7(2), 34-42.

Anuj Nautiyal, N.V. Madhav, S. Sharma R.K. Ojha, A. and Bhargava, S. (2015). Review on Nosocomial Infections. Carib. J .Sci Tech. 3: 781-788.

Atif A. A., Hani O., Alawayia M., Hassan A. M., Abdalla B. A.,Zein K., and Hassan S. H. (2000). Antimicrobial Agent Resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. *Am. J. Trop. Med. Hyg*., 63(5, 6), pp. 259–263.

Ayliffe G. A. J, 1970. Use of antibiotics and resistance. Geddes AM, William J.D, eds. Current Antibiotic Therapy. London: Churchill Livingstone, 53–60.

Baron, E. and Finegold, S. (1990). Bailey & Scotts Diagnostic Microbiology. 8th ed. Mosby. The C.V. Mosby Company, USA.

Bhan M. K., Bahl R. and Bhatnagar S. (2005) Typhoid and paratyphoid fever. *Lancet*, 366 (9487): 749–62.

Cheesbrough M. (2006). District Laboratory Practice in Tropical Countries. Cambridge University Press p. 434.

Chikere C. B, Chikere B. O., and Omoni VT (2008). Antibiogram of clinical isolates from a hospital in Nigeria. *Afr. J. Biotechnol*. 7 (24), 4359 -4363.

Collee, J. G.; Fraser, A. G.; Marmion, B. P. and Simmons, A. (1996) Mackie and McCartney, Practical Medical Microbiology, 14th Ed. Churchill Livingston, New York. 978 .

Courvalin P, Weber JT (2005). Antimicrobial drugs and resistance. *Emerg. Infect. Dis*., 11, 791-797.

Coyle MB (2005). Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology Press, Washington D.C. USA, p 25, 39

Debas G., Kibret M., Biadglegne F. & B. Abera. (2011). Prevalence and antimicrobial susceptibility patterns of *Shigella* Species at Felege Hiwot Referral Hospital , Northeast Ethiopia. *Ethiopia Medical Journal* 49(3), 55 - 60.

Eldaif, W. A., Omer A. and N. Saeed.2015. In-vitro antimicrobial susceptibility testing of bacteria causing diarrhea in children at Khartoum State-Sudan. *Indian J. Pharm. Biol. Res*. 3(1):32-35.

Farrar E. W., (1985). Antibiotic resistance in developing countries. *J. Infect Dis*. 152(6): 1103–1106.

Finch, R. G, (1998). Antibiotic resistance. *J Antimicrob. Chemother. 42: 125–128.*

Forbes, B. A., Sahm, D. F. and Weissfeld, A. S. (2007) Diagnostic Microbiology 12th Ed. Mobsy Elsevier, Houston Texas 324-329.

Godwin Wilson, Joshy M. Easow, Chiranjoy Mukhopadhyay and P.G. Shivananda.(2006). Isolation and antimicrobial susceptibility of *Shigella* from patients with acute gastroenteritis in western Nepal. *Indian J. Med. Res.* 123, 145-150.

Hussein, E. F. (2016). Isolation, Identification and Antibiotic Susceptibility of pathogenic Bacteria Isolated from Clinical Samples. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 11(4 II), 27-39.

Iffat J, Rubeena HM, Saeed A (2011). Antibiotic susceptibility patterns of bacterial isolates from patients admitted to a tertiary care hospital in Lahore. Biomedica J. 27:19-23.

Iroha R., Nwakeze E., Afiukwa N., UduIbiam O. Nwuzo C., Oji E. and N. Ngwu.2013. Antibiotic susceptibility patterns of bacterial isolates from hospitalized patients in Abakaliki. *International Res. J. of Basic and Clinical Studies* 1(4), 46-52.

Krishnan P, Stalin M and Balasubramanian S (2009) Changing trends in antimicrobial resistance of *Salmonella enterica sero* var. *typhi* and Salmonella enterica sero var. paratyphi A in Chennai. *Indian J. Pathol. Microbiol*, 52: 505-8.

Kunin C. M., Lipton H. L., Tupasi T., Sacks T., Scheckler WE, Jivani A., Goic A., Martin R. R., Guerrant RL, Thamlikitkul V, 1987. Social, behavioral and practical factors affecting antibiotic use worldwide: report of Task Force 4. Rev Infect. Dis. 9 suppl. 3:S270–S285.

Lovely A., Munir H. and Zakaria A. (2012). Present Status And Antibiotic Sensitivity Pattern Of Salmonella Typhi And S. Paratyphi In Different Age Group Hospitalized Patients In Dhaka City, Bangladesh. J. of Pharm. and Bio. Science 4(3), 27-30.

Martin, P, A, Lalitha MK, Semina N, Kronvall G, Guzman M, 1997. Reports on surveillance of antimicrobial resistance in individual countries. *Clin. Infect. Dis. 24 Suppl. 1*, S169–S175.

National Committee for Clinical Laboratory Standards (NCCLS) (2002). Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. NCCLS document M100-S12. PA, USA.

Okonko I. O., Donbraye-Emmanuel O. B., Ijandipe LA, Ogun AA, Adedeji, A. O. and Udeze, A. O. (2009a). Antibiotics sensitivity and resistance patterns of uro-pathogens to nitrofuration and nalidixic acid in pregnant women with urinary tract infections in Ibadan, Nigeria. *Middle –East J. Sc. Res.* 4(2), 105-109.

Okonko IO, Soleye FA, Amusan TA, Ogun AA, Ogunnusi TA, Ejembi J,Egun OC, Onajobi BI (2009b). Incidence of Multi-Drug Resistance (MDR). Organisms in Abeokuta, Southwestern Nigeria. *Global J. Pharmacol.* 3(2), 69-80.

Parry MF, 1989. Epidemiology and mechanism of antimicrobial resistance. Am. J. Inf. Control 17(5): 286–294.

Patrícia L. D., Caroline S. P., Lisandra D. R., Guilherme B. C., Roger T. F., Carolina P. S., Luiz M. F., Francisco J., Edilberto N. M, Teresa C. F. and Paula P. M.2017. Antimicrobial susceptibility profile of enterotoxigenic and enteropathogenic *Escherichia coli* isolates obtained from fecal specimens of children with acute diarrhea. *J. Bras. Patol. Med. Lab.*, 53(2), 115-118.

Power J. H. (2004). Antimicrobial drug development: The past, present and future. *Clin. Microbio. Infect*. 10(Suppl. 4): 23-31.

Rahal K, Wang F, Schindler J. Rowe B, Cookson B, Huovinen, P., Martin, A. Lalitha, M. K., Semiina, N., Kronvall, G. and Guzman, M. (1997).Reports on Surveillance of antimicrobial resistance in individual countries. *Clin. Infec. Dis. 24 Suppl*. 1, S169-S175.

Stella S., Moses B., Muinah F., Helen G., T., Emmanuel O., A., Kehinde A., A., Toun F., Rob P., Theresa A., H. and Henk S., L. (2011). Application of a Point-of-Care test for the Sero-diagnosis of Typhoid Fever in Nigeria and The Need for Improved Diagnostics. *J. of infect. Develop. Count.* 5(7), 520-526.

Tenvor FC, Hughes JM, 1996. The challenges of emerging infectious diseases: development and spread of multiply-resistant bacterial pathogens. *JAMA* 275(4), 300–304.

Udeze A. O. (2009a). Antibiotics Sensitivity and Resistance Patterns of Uropathogens to Nitrofurantoin and Nalidixic Acid in Pregnant Women with Urinary Tract Infections in Ibadan, Nigeria. *Middle-East J. Sci. Res.* 4 (2): 105-109.

Urassa W, Lyamuya E, Mhalu F, 1997. Recent trends on bacterial resistance to antibiotics. *East. Afr. Med. J*. 74(3), 129–133.

Wain, J.; Diep, T.S.; Ho, V.A.; M. Walsh,A; Hoa, N.T; M.Parry. C. and White, N. (1998). Quantitation of Bacteria in Blood of Typhoid Fever Patients and Relationship between Counts and Clinical Features Transmissibility, and Antibiotic Resistance. *J. of Clin. Microbiol*., 1683-168.