

Research Article

Prevalence and Antimicrobial Susceptibility Patterns of Enteric Gram Negative Bacteria in the Intensive Care Units of Kenyatta National Hospital

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Abstract: To investigate the prevalence of Enteric Gram negative bacteria and their antimicrobial susceptibility patterns in Intensive Care Units of Kenyatta National Hospital. No documented study has been done in Kenyatta National Hospital to determine the trends of antimicrobial susceptibility patterns in Intensive Care Units. The study was a laboratory-based study. All the clinical specimens received in the laboratory for culture and sensitivity from Intensive Care Units were subjected to the study. Isolation of Enteric gram-negative bacteria from clinical specimens and identification to the species level was performed by standard methods. Microbiology Laboratory, Kenyatta National Hospital, Kenya. The most prevalent microorganisms were found to be *Klebsiella pneumoniae* (10.5%), *Citrobacterfreundii* (8.6%), *Enterobacter spp* (5.2%), *Escherichia coli* (5.1%), *P. aeruginosa* (4.6%), *Proteus mirabilis* (3.2%) and *S.typhimurium* (0.14%). A percentage resistance of 10% or less was considered low and a percentage resistance of 50% and above was taken to be high. Moderate resistance was taken to be varying from 11 to 49%. *Citrobacterfreundii*, *Proteus mirabilis*, *E. coli* and *Enterobacter spp* showed high resistance to at least six antibiotics tested. *Klebsiella* and *Pseudomonas spp* showed high resistance to five and four antibiotics respectively. Moderate resistance was exhibited by all the six micro-organisms to an average of five antibiotics tested. Gram negative bacteria, specifically *Klebsiellapneumoniae.*, *Enterobacter spp.*, *Citrobacterfreundii.*, *E.coli*, *P. aeruginosa* and *Proteus mirabilis.* are prevalent in the Intensive Care Units of Kenyatta National Hospital. Isolated micro-organisms exhibited antimicrobial resistance to five commonly used antimicrobials.

Keywords: Antimicrobial resistance, critical care units, enteric gram negative bacteria, Kenyatta national hospital, prevalence

INTRODUCTION

Kenyatta National Hospital has four intensive care units namely, Critical Care Unit (CCU), Burns Unit, Renal Unit and Newborn Intensive Care Unit. Due to the important role played by these areas in the management of patients, constant assessment of infectious agents and possible development of resistance to available drugs is of utmost importance. Availability of such important data will help in the management of patients and lower possibility of cross-contamination as well as existence of drug resistant strains. Additionally this will lower the strain caused on the hospital facilities and scarce financial resources available by reducing the hospital stay by patients and cost of treatment (Pittet *et al.*, 1994).

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistant

elements, antibiotics promote the emergency of multi-drug resistant strains of bacteria; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The widespread use of antimicrobials for therapy or prophylaxis (including topical) is the major determinant of resistance. Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial becomes widely used, bacteria resistance to this drug eventually emerges and may spread in the health care setting. Many strains of *Pneumococci*, *Staphylococci*, *Enterococci* and *Tuberculosis* are currently resistant to most or all antimicrobials, which were once effective, Multi-resistant *Klebsiella* and *Pseudomonas* are prevalent in many hospitals. This problem is particularly critical in developing countries where more

expensive second-line antibiotics may not be available or affordable (DICNI, 1990).

The emergence and dissemination of resistant bacteria is a natural process in which bacteria get adapted to a hostile environment rich in antibacterial agents. However, it is not a phenomenon, which we cannot influence. This is evident from the fact that there is tremendous difference in the prevalence of resistance in hospitals and communities throughout the world. The advent of penicillin in 1944 suggested the defeat of infection. Within a few years, resistant *Staphylococcus aureus* burst the euphoric bubble. β -lactam antimicrobial agents are still most widely used and so is the resistance against them. The most important mechanism of resistance to β -lactam agents is the production of the enzyme β -lactamase, which destroys the β -lactam ring. They are produced constitutively or as induced enzymes. It was believed that Cephalosporins are relatively immune to β -lactamases. It was disappointing when in *Klebsiella pneumoniae*. Plasmid mediated resistance was found against broad-spectrum cephalosporin. The resistance is attributed to novel β -lactamase enzymes known as extended spectrum beta lactamases (ESBLs) (Cohen, 1992).

Due to emergence of multidrug resistant bugs, the need to keep track of antimicrobial susceptibility trends has become mandatory. Close co-operation between pharmacists, clinicians and microbiologists will enable the hospital to come up with a drug policy that takes into account the cost-effectiveness of the therapeutic agents of proven efficacy (Tenova and Hughes, 1996).

Kenyatta National Hospital (KNH) spends huge sums of money in management of Intensive Care Unit patients colonized or infected with hospital strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Acinetobacter*spp. that are resistant to nearly all routinely used antibiotics. These multidrug resistant (MDR) bacteria are normally isolated from tracheal aspirates of patients on mechanical respirators. Cases of multi-drug resistant *Klebsiella pneumoniae* have also been observed in newborn units (Mayon-White *et al.*, 1988).

MATERIALS AND METHODS

Study area/study population: The study was carried out in the department of Microbiology, Kenyatta National Hospital, Kenya, between August 2015 and January 2016. All the clinical samples received in the department for culture and sensitivity from Intensive Care Units was subjected to the study. The total number of specimens was 675, (from Critical Care Unit (CCU) -301, Burns Unit (BU)-93, Renal Unit (R/U)-48 and Newborn Intensive Care Unit (NICU) -233). The specimens were blood, urine, tracheal aspirate and pus swabs. The total number of bacterial organisms isolated

were 333 (*E. coli*-38, *Proteus mirabilis*-20, *Citrobacter freundii*-50, *Klebsiella pneumoniae*-72, *Enterobacter spp*s-38 and *Pseudomonas aeruginosa*-61).

Ethical consideration: Ethical approval for the study was granted by the Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (KNH/UON-ERC).

Sample collection: All specimens were collected and transported in accordance with the established Standard Operating Procedures (SOPS).

Sample analysis: A representative sample of the specimen was Gram stained and examined microscopically for the characteristic features and identification of the enteric gram-negative bacteria. A representative sample of the specimen was inoculated onto the culture media (Blood agar, MacConkey, Xylose lysine deoxycholate, Salmonella Shigella agar and Nutrient agar, appropriately) and incubated at 37°C for at least 12 h. Biochemical tests were done to differentiate the isolated microorganisms. They included; Kligler iron agar, citrate test, Catalase test, Motility indole urea test and Oxidase test. The antimicrobial sensitivity testing was done by Kirby-Bauer disc diffusion method standardized as per NCCLS (Washington, 1991). Antibiotics were selected according to WHO model list of essential drugs (Jones *et al.*, 1997). For internal quality control *E.coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) strains was used (NCCLS, 2008).

Internal quality control: The following measures were taken to reduce pre-analytical errors: avoided sample contamination, proper specimen labelling, Proper specimen handling and storage, used the recommended media at all times and used the recommended laboratory procedures. Controls were provided by a series of reference strains, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213 for dilution test; ATCC 25923 for disk test), *Streptococcus faecalis* (ATCC 29212), *Haemophilus influenza* (ATCC 49247) and *Neisseria gonorrhoeae* (ATCC 49226), for which expected results were established. These reference strains were available from the American Type Culture collection in Washington DC, or from various commercial sources. The ideal control strains have susceptibility end points in the mid-range of antimicrobial concentrations tested and have minimal tendencies to change susceptibility patterns over time.

Data management and analysis: The software (WHONET) used to analyse the data was available through World Health Organization (WHO). The

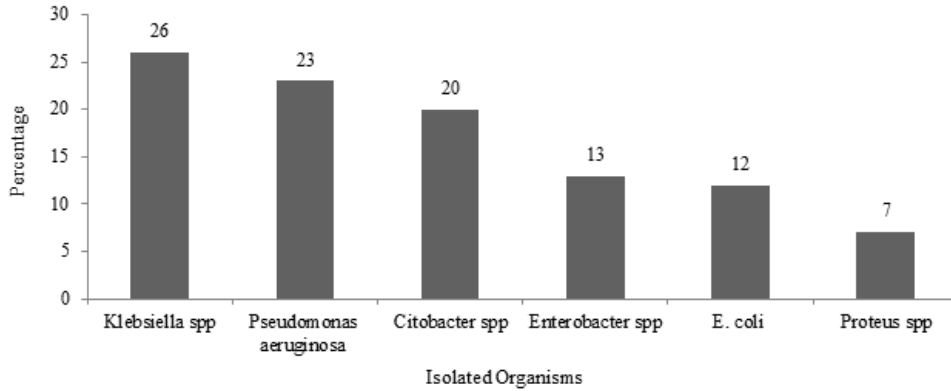


Fig. 1: Prevalence of Gram negative bacteria in all ICUs

information entered into the computer system for the purpose of data analysis included: Laboratory registration number, inpatient/outpatient number, age of the patient and sex of the patient, preliminary diagnosis, type of specimen, organism isolated and sensitivity pattern.

RESULTS

Figure 1 shows the prevalence of Gram negative bacteria in all the Intensive Care Units of Kenyatta National Hospital. *Klebsiella pneumoniae* had the highest prevalence of 26% followed by *Citobacter spp.* (20%), *Enterobacter spp.* (13%), *E. coli* (12%), *Pseudomonas aeruginosa* (23%) and *Proteus mirabilis* (7%)

Figure 2 shows the susceptibility pattern of *Citrobacterfreundii*. The isolates showed high resistance to ampicillin (65.8%), Cefuroxime (76.9%), Ceftaxidime (71%), Ceftriazone (78.6%) and *Gentamycin* (77.8%). Moderate resistance was noted with Tazobactam (33.3%), *Ciprofloxacin* (25%), *Imipenem* (15%) and *Meropenem* showed the lowest resistance (2.8%).

Figure 3 shows the susceptibility pattern of *Proteus mirabilis*. The highest resistance was noted with *Amoxicillin* (63.6%, *Cefuroxime* (68.8%) and *Gentamicin* (62.5%). Moderate resistance occurred with Ceftazidime (40%) and *Ciprofloxacin* (20%). Lowest resistance was noted with *Imipenem* (8.3%) and *Meropenem* (5.9%).

Figure 4 shows Susceptibility patterns of *Klebsiellapneumoniae*. The isolates showed the highest resistance with *Cefuroxime* (85.8%), *Gentamicin* (68.2%), *Amoxicillin* (64.8%) *Ceftazidime* (52.2%), *Nalidixic* (50%). *Amikacin*, *Levofloxacin* and *Ciprofloxacin* showed moderate resistance (35.3%, 30% and 29% respectively). No resistance was noted with *Imipenem* and *Meropenem*.

Figure 5 shows the susceptibility pattern of *E. coli*. The isolates showed high resistance to *Nalidixic*

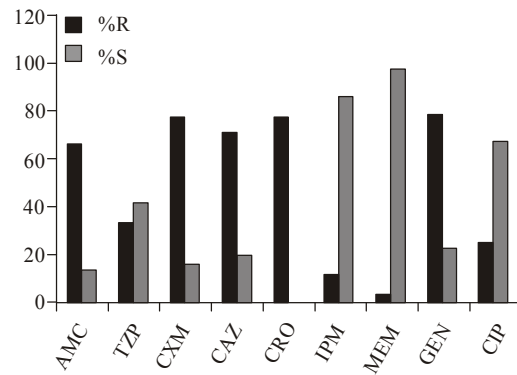


Fig. 2: Susceptibility patterns of *Citrobacterfreundii*

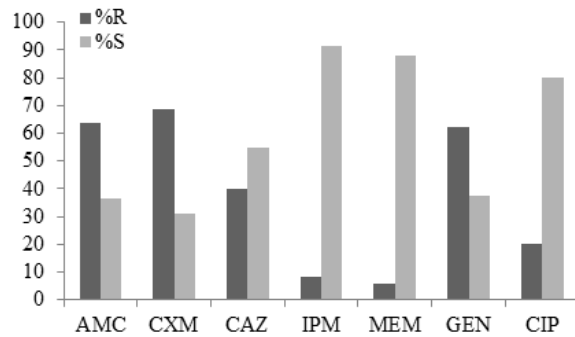


Fig. 3: Susceptibility patterns of *Proteus mirabilis*

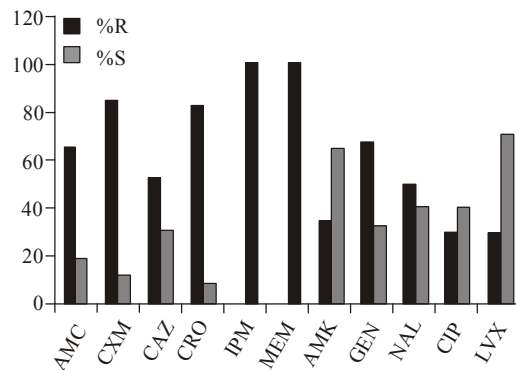


Fig. 4: Susceptibility patterns of *Klebsiella pneumoniae*

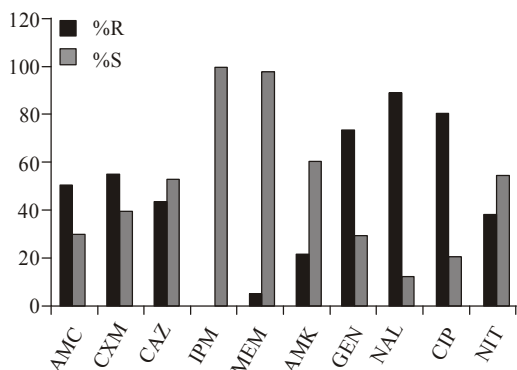


Fig. 5: Susceptibility patterns of *E. coli*

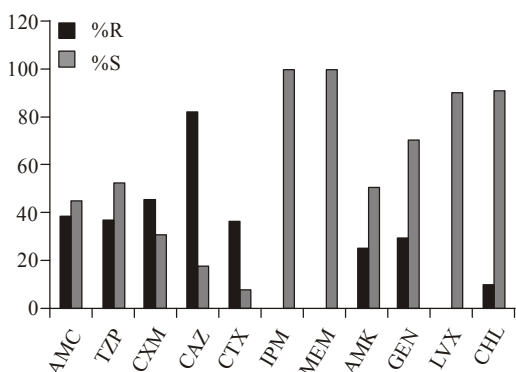


Fig. 6: Susceptibility patterns of *Enterobacter spp.*

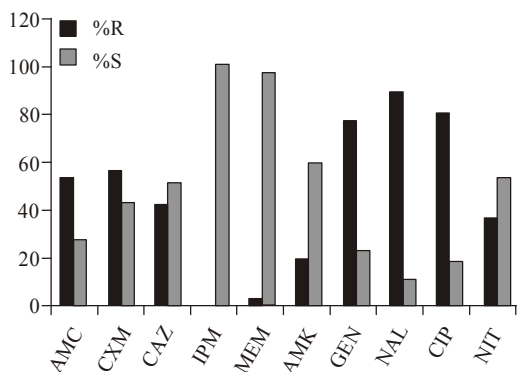


Fig. 7: Susceptibility patterns of *P. aeruginosa*

(88.2%), Ciprofloxacin (80%), Gentamicin (72.8%), Cefuroxime (56.2%) and Amoxicillin (50%). Moderate resistance was noted with Ceftazidime (41.7%), Nitrofurantoin (35.3%) and Amikacin (20%). The lowest resistance was noted with Meropenem (3.7%) and Imipenem (0%).

Figure 6 shows the susceptibility pattern of *Enterobacter spp.* Ceftazidime showed the highest resistance (81.8%). Moderate resistance was noted with Cefuroxime (45%), Amoxicilline (37.9%), Tazobactam (36.8%), Ceftriazone (36.4%), Gentamicin (30%) and Amikacin (25%). Lowest resistance was noted with

Chloramphenicol (10%), Imipenem (0%), Meropenem (0%) and Levofloxacin (0%).

Figure 7 shows Susceptibility patterns of *P. Aeruginosa*. The highest resistance was noted with Nalidixic (88.2%), Ciprofloxacin (80%), Gentamicin (76.5%), Cefuroxime (54.8%) and Amoxicillin (51.7%). Moderate resistance was noted with Ceftazidime (42.9%), Nitrofurantoin (35.3%) and Amikacin (20%). Meropenem and Imipenem showed the lowest resistance (3.8% and 0%, respectively).

DISCUSSION

In this study, *Klebsiella pneumoniae* were isolated in all the intensive care units. Higher percentage was noted in the critical care unit (16.5%). The isolates were recovered from tracheal aspirates (probably due to ventilator related infections of the upper respiratory tract), urine due to catheterization and blood due to septicemia and finally pus swab due to wound infection. *P. aeruginosa* was isolated in Critical Care Unit (CCU) and Burns Unit with the prevalence rate of 10.3 and 33.7% respectively. Tracheal aspirates, urine and pus gave the highest share of the isolates. This is attributable to the ventilator and catheter insertion during CCU patients' management as well as secondary infection in burn wounds. *Citrobacterfreundii*. were isolated in all the four Intensive Care Units. In Burns Unit it had 12.2% prevalence due to secondary infections in burn wounds. Their occurrence in CCU and NICU was also high due to upper respiratory infections and septicemia, respectively. *Proteus mirabilis* had the highest percentage in Burns Unit where pus swab specimens were obtained. This was as a result of its implication in wound infections and septicemia. *E. coli* was isolated in all units especially CCU. Most isolates were obtained from urine specimens due to urinary tract infections associated with catheterization.

In terms of antimicrobial sensitivity testing, up to 15 drugs were used to carry out the assay. It was noted that isolates had varied susceptibility patterns ranging from susceptible, moderate resistance to high resistance. *Citrobacterfreundii* were resistance to 5 drugs out of the total of 9 drugs tested. It showed moderate resistance to 3 drugs and it was highly susceptible to 1 drug. *Proteus mirabilis* was susceptible to 2 drugs and showed moderate resistance to 2 drugs. It showed high resistance to 3 drugs. A total of 7 drugs were involved. *Klebsiella pneumoniae* was susceptible to 2 drugs out of the 11 drugs tested. Moderate resistance was noted against 3 drugs tested and high resistance to 6 drugs. *E. coli* was susceptible to 2 drugs, moderately resistant to 3 drugs and highly resistant to 5 drugs. *Enterobacter spp.* was susceptible to 4 drugs, moderately resistance to 6 drugs and highly resistant to 1 drug. *P. aeruginosa* showed sensitivity to 2 drugs and

moderately resistant to 4 drugs and highly resistant to 5 drugs.

Nema *et al.* (1997) found that 73-99% of gram negative isolates were resistant to common antibiotics like ampicillin, chloramphenicol, cotrimoxazole and first generation cephalosporins. The resistance to gentamicin and ciprofloxacin ranged from 53 to 79%. Resistance to amikacin, netilmicin and third generation cephalosporins (3GC) ranged from 30 to 73% (Nema *et al.*, 1997). Valdivieso *et al.* (1999) conducted a twelve month study in 11 Chilean hospitals on urinary isolates. They found that 65% strains of *E.coli* were resistant to ampicillin, 43% to cotrimoxazole, 9% to ceftazidime, 4.2% to gentamicin, 5.6% to ciprofloxacin, 4.3% to nitrofurantoin and 1.3% to amikacin. Jones *et al.* (1997) found 23.8% isolates of *Klebsiella pneumoniae*. to be resistant to ceftazidime whereas Bantar *et al.* (2000) found 48% isolates to be resistant to third generation cephalosporins. 71% isolates of *Klebsiella pneumoniae*. from blood samples have been reported to be resistant to third generation cephalosporins (Valdivieso *et al.*, 1999).

CONCLUSION

The outcome of the study confirmed that Gram negative bacteria, specifically *Klebsiellapneumoniae*., *Enterobacter spp.*, *Citrobacterfreundii*., *E.coli*, *P. aeruginosa* and *Proteus mirabilis*. are prevalent in the Intensive Care Units of Kenyatta National Hospital and were resistant to some of the commonly used antibiotics.

RECOMMENDATIONS

It is therefore suggested that the use of third generation cephalosprins be used only in dire emergencies. In routine, specific therapy should be sought after antimicrobial sensitivity testing.

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