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PANDRUG- RESISTANT *KLEBSIELLA SPECIE*: CASE REPORT OF AN UNUSUAL ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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ABSTRACT
Klebsiella species over the past decade have continued to pose difficulties in its treatment with antibiotics due to its high resistance across the globe. This study was based on observation of resistance pattern of the bacterium in a new university teaching hospital in West Africa.This study was carried out to ascertain the scope of antimicrobial resistance of a *Klebsiella specie* from urine sample of a patient. Urine sample from a male patient was collected into sterile universal bottle, transported to the laboratory and processed using standard laboratory methods. These include macroscopy, wet preparation, Gram stain, inoculation to culture media and incubation, and biochemical tests where *Klebsiella* was identified. Antibacterial susceptibility test was carried out on Mueller-Hinton agar using modified Kirby-Bauer disk diffusion method and results interpreted as either resistant or sensitive. The *Klebsiella spp* was resistant to Ampicillin, Ciprofloxacin, Erythromycin, Streptomycin, Cefuroxime, Gentamycin, Ceftriaxone, Perfloxacin, Tetracycline, Amoxycillin, Clavulanate+Amoxicillin (Amoxiclav) and Co-trimoxazole but sensitive to only clindamycin. Empirical treatment of infections with antibiotics should generally be scaled down in the tropics and sale and intake of antibiotics at community level properly controlled and legislated. Also the option of introducing automated and semi automated techniques with advantage of shortened time in sensitivity testing be explored for rather definitive treatment of infections.

INTRODUCTION

Klebsiellae belong to the family Enterobacteriaceae and are commonly associated with both community-acquired and nosocomial infections among humans such as septicaemia, pneumonia, wound sepsis, and meningitis[1]. There have generally been mixed reports across the globe about the antimicrobial susceptibility pattern of the organism but with a general ly agreed pattern of an increasing antimicrobial resistance of *Klebsiella species* over the past decade[2-3]. While report from Lebanon quoted *K. pneumonia* to have multidrug resistance (MDR) of less than 25%[4], that from Turkey reported a MDR of over 80% and extended-spectrum beta-Lactamase carriage[5]. And report from China and Bahrain showed a wide range of MDR among the antimicrobials [6,7]. These varied susceptibility patterns of *K. spp.* across the globe have often caused therapeutic failures with grave consequences [8]. We report here another abnormal antimicrobial susceptibility pattern of a *K. spp.* isolate at our teaching hospital and further discussions.

MATERIALS AND METHODS

Procedure

The study carried out at BSUTH was based on observations of antimicrobial susceptibility reports of *Klebsiella spp.* within a span of six months (July-December) in 2014**.** Urine was obtained from the patient through normal voiding. The urine sample (approx.15 mls)

which was collected into universal specimen bottle was transported to the Microbiology laboratory within 20 minutes of collection and was processed immediately on arrival. Macroscopy of the urine sample was carried out by viewing it through a well lit surface. Wet preparation was carried out by placing three loopfuls of well mixed urine on a slide and covered with a cover glass. This was examined with both X10 and X40 with condenser iris closed sufficiently for optimum contrast.

With the aid of a sterile wire loop, a loopful of well mixed urine was collected and a smear made on a clean glass slide. This was air dried, fixed by passing it over Bunsen flame twice and subsequently stained by Gram's Method using crystal violet, lugol's iodine, acetone and safranin. The stained smear was air-dried and examined under X100 objective oil immersion [9,10].

The urine sample was inoculated into cystein lactose electrolyte deficient medium (CLED), Blood agar and MacConkey agar solid media and incubated overnight at 36.5^{O}C aerobically. From the culture plates Gram stain was repeated from the colonies.

The following biochemical tests were carried out: lactose, glucose and sucrose fermentation, oxidase test, urease and citrate test, indole and motility tests.

Sub-culture of like colonies into peptone water broth was done the following day and incubated at 36.5^{O}C for two hours while the broth was standardized using 0.5 Mc Farland's turbidity standard. Antimicrobial susceptibility of the isolate was carried out using modified Kirby-Bauer's disk diffusion method. Sterile cotton wool swab stick was dipped into standardized culture broth; excess fluid was drained and the moist cotton rubbed uniformly on prepared Mueller-Hinton plate agar. Antibiotic disks of 6 millimetres diameters were then placed on the inoculated agar plates and incubated overnight at 36.5^{O}C aerobically and read the following day. The diameters of zones of inhibition of the various antibiotics were interpreted by comparing them with standard sensitivity tables. Antibiotics used along with the corresponding disk content of antibiotics were as follows: Ampicillin (10µg), Amoxiclav {Amoxicillin+Clavulanic acid(20µg)}, Gentamicin (10µg), Ceftriaxone(30µg), Ciprofloxacin (5µg), Perfloxacin (5µg), Erythromycin (10µg), Streptomycin (10µg), Cefuroxime (30µg), Tetracycline (20µg), Amoxicillin (10µg), Co-trimoxazole (10µg) and Clindamycin (10µg) [9,10]. The Result was interpreted using simple descriptive methods.

RESULTS

The isolate was identified as *Klebsiella. spp.* based on the following characteristics: Gram negative bacillus, oxidase negative, non motile, indole negative, non Hydrogen sulfide producing, fermenting both lactose sucrose and glucose with gas production, and is citrate and urease positive. The isolate was resistant to Ampicillin, Ciprofloxacin, Erythromycin, Streptomycin, Cefuroxime, Gentamycin, Ceftriaxone, Perfloxacin, Tetracycline, Amoxycillin, Clavulanate + Amoxicillin (Amoxiclav) and Co-trimoxazole. The *K. pneumoniae* was only susceptible to Clindamycin (Table 1).

Table 1. Antimicrobial susceptibility profile of *Klebsiella spp.* from urine of a male patient with urethral stricture at Benue State University Teaching Hospital, Makurdi Nigeria

Antimicrobial	Susceptibility Profile
Ampicillin	Resiatant
Ciprofloxacin	Resiatant
Erythromycin	Resiatant
Streptomycin	Resiatant
Cefuroxime	Resiatant
Gentamycin	Resiatant
Ceftriaxone	Resiatant
Perfloxacin	Resiatant
Tetracycline	Resiatant
Amoxicillin	Resiatant
Clavulanate+Amoxicillin (Amoxiclav)	Resiatant
Co-trimoxazole	Resiatant
Clindamycin	Susceptible

DISCUSSION

Among the 13 antimicrobials tested against the *Klebsiella spp*. isolate at BSUTH Makurdi, only Clindamycin was found to be active while the rest-Ampicillin, Ciprofloxacin, Erythromycin, Streptomycin, Cefuroxime, Gentamycin, Ceftriaxone, Perfloxacin, Tetracycline, Amoxycillin, Clavulanate + Amoxicillin (Amoxiclav) and Co-trimoxazole were found to be inactive. Findings from this study create additional challenge among physicians and other health personnel during empirical prescription of those antibiotics especially in life threatening conditions where antibiotic failure is not a tolerable outcome [11,12]. The finding from the present study probably stresses the fact that even though antibiotic pressure from health settings play significant role in

nosocomial anti-microbial resistance, in newer health settings like ours with short duration of this pressure, resistance is still pronounced. This may be attributed to the natural selection process among bacteria going on at the community level as well [13]. The isolation of *Klebsiella spp*. from community acquired infections in our laboratory which were resistant to four, five, and more than six antibiotics further strengthens this assertion.

The contribution of antibiotic pressure at the community level within Makurdi city and also several other communities across Nigeria have probably continued to propel this wave of resistance in the city and indeed the country as a whole. Unconventional practices such as: free prescription of antibiotics at pharmacy shops, prescriptions at private medical laboratories by various paramedical staff, issuance of erroneous sensitivity reports at private laboratories, and hawking of antibiotics at market places and inside public vehicles (buses and trains) and self-medications all contribute to the buildup of antibiotic pressure at the community level [14,15]. This scenario appear to be a common practice in most communities in sub-saharan Africa as well as other resource restrained parts of the world[16-19]. Proper regulation of these practices would adequately reduce the spate of resistance in the community and her health settings.

Klebsiella spp. have over the last decade increasingly posed serious clinical challenges from treatment of its associated infections in health settings worldwide [18-20]. The organism is one of the components of the 'superbug' and belong to the ESKAPE group, the other members being: *Enterococcus faecium, Staphylococcus aureus, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp*.

These bacteria along with *Klebsiella pneumonie* have been found to be resistant to virtually all the antibiotics on the shelf of a pharmacy shop and have been associated with severe infections with fatal outcomes [21,22]. The *Klebsiella spp*. isolated in the present study was resistant to second and third generation cephalosporins, second and third generation quinolones, fortified forms of penicillins, and other generally potent aminoglycosides. The general tendency to prescribe or recommend the intake of these drugs while waiting for a valid antimicrobial susceptibility report should be borne out of the fact that they can equally fail like the lower drugs [23,24].

Real time automated susceptibility testing methods should probably be considered as an alternative so as to considerably shorten the time of prescribing definitive antibiotic after the patient is seen especially where the life of the patient is time bound. Also hospitals in this region and probably other parts of sub-saharan Africa should as a matter of policy issue weekly or monthly antibiograms on antibiotics in common use in the locality so as to guide physicians on the choice of antibacterial agents for empirical treatment [25,26].

CONCLUSION

The isolation of highly resistant *Klebsiella spp*. in this centre poses more challenge to the fact that any antibacterial in the hospital has the possibility of being inactive and this should be acknowledged. Proper legislation should be put in place and enforced to closely monitor antibiotic prescriptions and intakes both within and outside established health settings. Also more advanced antimicrobial susceptibility methods with comparative advantage of reducing time of results should be considered so as to reduce incidences of empirical treatment.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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