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Isolation, Phenotypic Characterization, Antimicrobial Resistance and Susceptibility of ESBL Producing-*Klebsiella pneumoniae* from Urine Specimen Collected from Patients in Maiduguri

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Abstract: *Klebsiella pneumoniae is one of the Enterobacteriaceae strains that can produce Extended-Spectrum Beta-Lactamases Enzymes (ESBLs) and become highly effective against different Beta-lactams antimicrobials. It is associated with different diseases which can cause high mortality and morbidity. This study aims to elaborate on the isolation, phenotypic characterization, antimicrobial resistance and susceptibility of ESBL Producing-K. pneumoniae from urine specimen collected from patients in Maiduguri. The study was Hospital-based, descriptive, and cross-sectional in design. Two hundred and twenty (220) clinical samples were collected for this study from both in and outpatients with clinical symptoms of urinary tract infections attending Umaru Shehu Ultra-Modern Hospitals Hospital and State Specialist hospital Maiduguri respectively. There is no significant association between hospitals and the status of the ESBL Producing-K. pneumoniae (P = 0.2789; DF = 1; Chi-squared = 1.172). The results obtained from urine culture revealed 122 (55.45%) positive and 98 (44.55%) negative samples of lactose fermenting organisms from both State Specialist Hospital Maiduguri and Umaru Shehu Ultra-Modern Hospital, out of which, 65 (29.55%) were positive and 45 (29.55%) were negative from State Specialist Hospital Maiduguri and 57(25.91%) were positive and 53(24.09%) were negative from Umaru Shehu Ultra-Modern Hospital. A total of 19 (8.46%) positive and 46 (20.91%) negative samples were K. pneumoniae and these were obtained from State Specialist Hospital Maiduguri and 11(5%) positive and 46(20.91%) negative samples of K. pneumoniae were obtained from Umaru Shehu Ultra-Modern Hospital. All the positive ESBL samples were resistant to all the antimicrobials with the exception of Amoxiclav and Imipenem. Furthermore, the non ESBL Producing-K. pneumoniae were all susceptible to all the antimicrobials except Ampicillin which was resistant. Therefore, there is need to use more antibiotic and antimicrobial agents. It is likewise imperative to identify the resistance genes associated with ESBL producing K. pneumoniae and the virulence genes associated with the ESBL genes.*

Keywords: *lactose-fermenting, ESBL-producing Klebsiella pneumoniae, In and Out patient, Antimicrobial agents, Maiduguri.*

Introductions

Klebsiella pneumoniae is one of the most significant multidrug-resistant (MDR) opportunistic Gram-negative bacteria. It is associated with different diseases which can cause high mortality and morbidity due to nosocomial and non-hospital acquired infections such as pneumonia, urinary tract infection (UTI), burns infections, and bacteraemia (Zhong *et al.,* 2013).

In recent times, *K. pneumoniae* became more resistant to antimicrobial mainly to third-generation cephalosporins. Many studies focused on the isolation of this pathogen from patients infected with different infections (Chili *et al.,* 2016; Singh *et al.,* 2017; and Karlowsky *et al.,* 2017).

K. pneumoniae is one of the Enterobacteriaceae strains that can produce Extended-Spectrum Beta-Lactamases Enzymes (ESBLs) and become highly effective against different Beta-lactams antimicrobials. These enzymes have the ability to hydrolyse these cephalosporins compromising the efficacy of these antibiotics, ESBLs-producing bacteria are resistant to various antimicrobial classes, leading to difficult-to-treat diseases called multi-drug resistance (MDR) (Nathisuwan *et al.,* 2001). Multidrug-resistant bacteria and ESBL producing *K. pneumoniae and* other Gram-negative bacteria have worldwide distribution with high degree of prevalence in both hospitals and communities (Leverstein-van *et al.,* 2002, Aljanaby, 2013, Legese *et al*., 2017). Extended spectrum beta lactamases are plasmid-mediated enzymes that are capable of conferring resistance to penicillins, first, second and third generations cephalosporins and carbapanems (Johann *et al.,* 2008). ESBL producing *K. pneumoniae* strains isolated from both in and outpatients can cause treatment failure with different antimicrobial therapy such as beta-lactams, cephalosporins, aminoglycosides and others (Kim *et al*., 2016; Tang *et al*., 2017). The ESBL are enzymes produced by different types of bacterial species as a means for defence against *β. Lactam* drugs with the genes encoding for those enzymes being mainly located on mobile genetic elements (Pfeifer *et al.,* 2016). The rapid emergence of ESBL producing Gram-negative bacteria like *K*. *pneumoniae* has significantly increased the risk of developing serious nosocomial infections worldwide (Brinkworth *et al.,* 2015; Latifpour *et al.,* 2016).

K. pneumonia has recently gained recognition as an infectious agent due to rise in the number of severe infections. These pathogens showed more resistance in response to treatment of pneumonia among neonates, elderly and immune-suppressed individuals within the healthcare-associated settings (Paczusu *et al.,* 2016; Quan *et al.,* 2016). Treatment of these infections depends heavily on effective antimicrobial therapy and delaying treatment may lead to a higher mortality ratio. Therefore, the presence of MDR genes in the infecting pathogen could negatively affect the treatment outcome (Lin *et al.,* 2016).

The prevalence varies worldwide and in closely related regions. In Nigeria, a study conducted by Yusha'u *et al* 2010 in Kano revealed 9.25% ESBLs production amongst isolates of *Enterobacteriaceae.* Also, a study conducted by Oluwe *et al.,* 2010, on the determination of ESBL prevalence recorded 5% prevalence of *K. pnuemoniae* and 2.5% prevalence of *Escherichia*

coli. There is no routine laboratory detection of ESBL producing isolates in most health care facilities and due to the high treatment failure associated with ESBL-producing organisms, it is imperative to ascertain the phenotypic characterization, their antimicrobial resistance profile and select the most appropriate drug for managing ESBL infection in Maiduguri Metropolis.

2 Materials and Methods

Study Area and Period.

The study was conducted at Maiduguri from January to March, 2022. Maiduguri which is located in the capital city of Borno stated.

Research Ethics and patient consent

Ethical clearance and protocol will be obtained from State Specialist Hospital and Umaru Shehu Ultra Model Hospital Maiduguri to enable the collection of samples. Patient consent was requested to collect swabs from wound.

Sampling Method.

A non-probability |(convenient) purposive type of sampling method was used.

Study Design

The study was Hospital-based, descriptive, and cross-sectional in design.

Sample Collection and Transportation: Two hundred and twenty (220) clinical samples were collected for this study. One hundred and ten (110) urine samples were collected were collected from both in and outpatients with clinical symptoms (Urinary Tract Infections UTIs) attending Umaru Shehu Ultra-Modern Hospitals (USUMH) Hospital and State Specialist hospital Maiduguri (SSHM) respectively.

Urine samples were collected in a well labelled universal containers indicating the source of the sample that is the Hospital in which the samples were collected. Samples were immediately transported to the laboratory for bacteriological procedures and analysis.

Bacterial Isolation

The urine sampled was inoculated on MacConkey agar (Himedia- India) surface and incubated aerobically over night at 37^0C. All isolates were preliminarily screen by their colony, morphology, colour (pinkish mucoid colonies) and gram staining techniques (Gram negative rod shaped, non-capsulated and non-sporing). Further identification included Biochemical test methyl red negative and indole negative, citrate positive, and non-motile. In addition, all *K. pneumoniae* isolates were streaked and inoculated on Hicrome selective *Klebsiella pneumoniae* agar (Himedia-media) which was used for final identification of *K. pneumoniae*

ESBL Detection Methods: Hicrome selective *Klebsiella pneumoniae* agar (Himedia) was used for the primary isolation of ESBL *K. pneumonia* prior to confirmatory screening as per clinical and laboratory standard institute guidelines 2014 (|CLSI, 2014|)

ESBL Screening and Confirmation

The ESBL test screening test was performed by the standard disk diffusion method using

cefotaxime (30µg), ceftriaxone(30µg) and ceftazidine (30µg) (Oxoid, UK). More than one antibiotic disk was used for screening to improve the sensitivity of ESBLs detection as per CLSI standard procedure (CLSI, 2014). Pure isolates were suspended into normal saline and the turbidity of the suspension was adjusted at 0.5 MacFarland's standard. The suspension was then inoculated onto Mueller-Hinton Agar (Himedia-India) with a sterile swab. Cefriaxone (30µg), ceftriaxone(30µg) and ceftazidine (30µg) were placed at a distance of 20mm and incubated at 37^0C overnight (16-15 hours). The isolates that were less sensitive to cefriaxone, (inhibitory zone ≤ 23mm) cefotaxime (inhibitory zone ≤ 27mm) and ceftazidine (inhibitory zone ≤ 22mm) around the disc were suspected to be ESBL-producing (CLSI, 2014). For the confirmatory test, the suspected ESBL-producing *K. pneumoniae*, a double disc synergy method on Mueller-Hinton agar was done according to 2014 CLSI guidelines. used to sscreened for ESBL production using the modified Kirby Bauer method. A disk of amoxicillin/clavulanic acid (20/10 µg) was placed at the middle of Mueller-Hinton agar plate and then ceftazidime and (30µg), cefotaxime (30µg) were placed at a distance of 20mm putting the amoxicillin/clavulanic acid in the middle of the plate. The inoculations were then incubated at 37^0C for 24 hours after which it was examined for an expansion of inhibition zone of the axyimino-β-lactamscaused by the synergy of clavulanate in the amoxicillin-clavulanate disk which was interpreted as a positive ESBL production.

Results

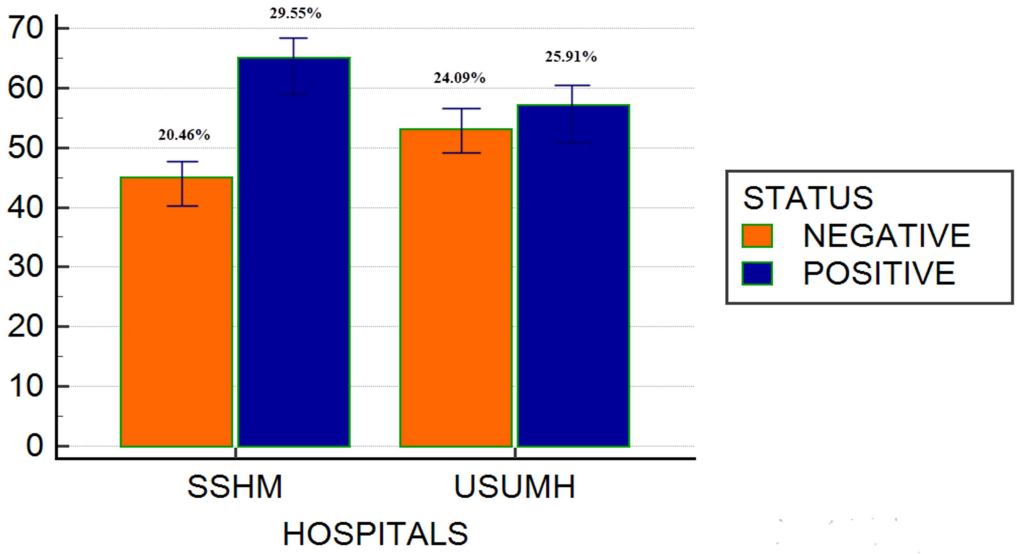

Figure 1: Growth of lactose fermenting organisms on MacConkey Agar

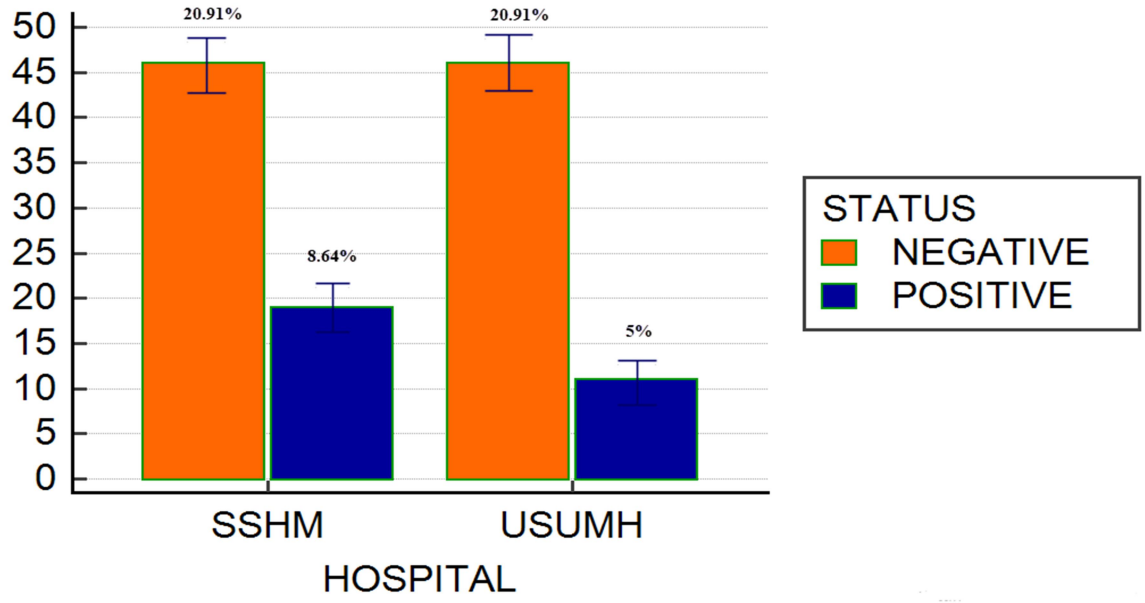

Figure 2: Isolation of *K. pneumoniae* from positive lactose fermenting organisms

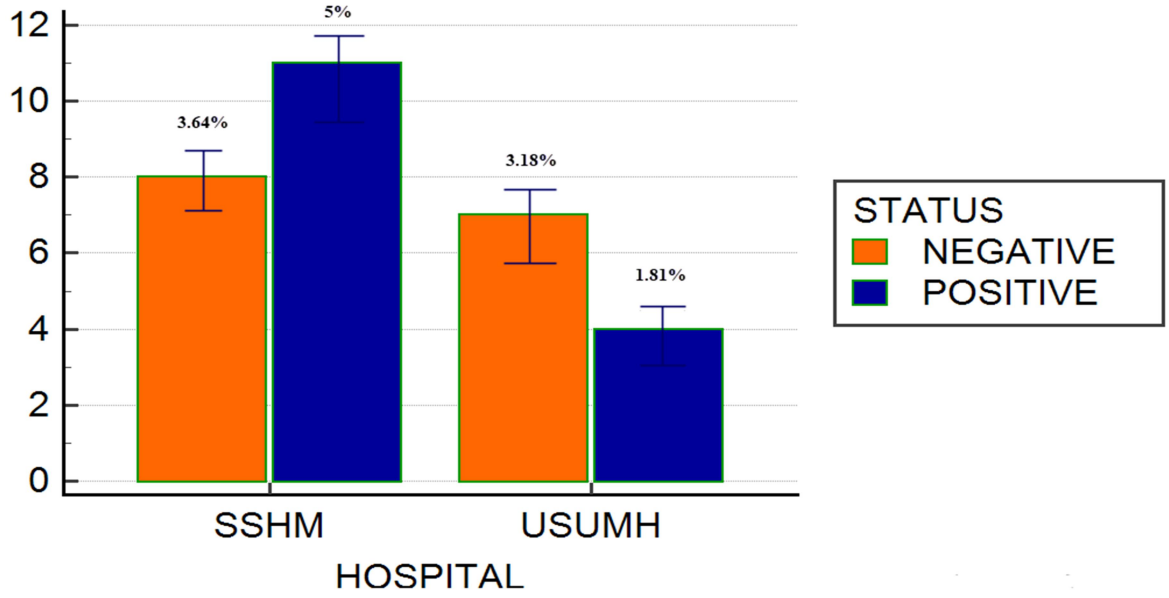

Figure 3: Positive ESBLs Producing-*K. pneumoniae*

The results obtained from urine culture revealed 122 (55.45%) positive and 98 (44.55%) negative samples of lactose fermenting organisms from both State Specialist Hospital Maiduguri (SSHM) and Umaru Shehu Ultra-Modern Hospital (USUMH), out of which, 65 (29.55%) were positive and 45 (29.55%) were negative from SSHM and 57(25.91%) were positive and 53(24.09%) were negative from USUMH. A total of 19 (8.46%) positive and 46 (20.91%) negative samples were *K. pneumoniae* and these were obtained from SSHM and 11(5%) positive and 46(20.91%) negative samples of *K. pneumoniae* were obtained from USUMH.

Table 1: Chi-Squared test for the growth of lactose fermenting organisms on MacConkey Agar

Chi-squared test

Chi-squared	1.172
DF	1
Significance level	P = 0.2789
Contingency coefficient	0.073

There is no significant association between hospitals and the status of the lactose fermenting organisms (P = 0.2789; DF = 1; Chi-squared = 1.172).

Table 2: Chi-Squared test for the isolation of *K. pneumoniae* from positive lactose fermenting organisms

Chi-squared test

Chi-squared	1.602
DF	1
Significance level	P = 0.2056
Contingency coefficient	0.114

There is no significant association between hospitals and the status of the *Klebsiella pneumoniae* (P = 0.2056; DF = 1; Chi-squared = 1.602).

Table 3: Chi-Squared test for the Positive ESBLs Producing-*K. pneumoniae*

Chi-squared test

Chi-squared	1.249
DF	1
Significance level	P = 0.2638
Contingency coefficient	0.200

There is no significant association between hospitals and the status of the *Klebsiella pneumoniae* (P = 0.2638; DF = 1; Chi-squared = 1.249).

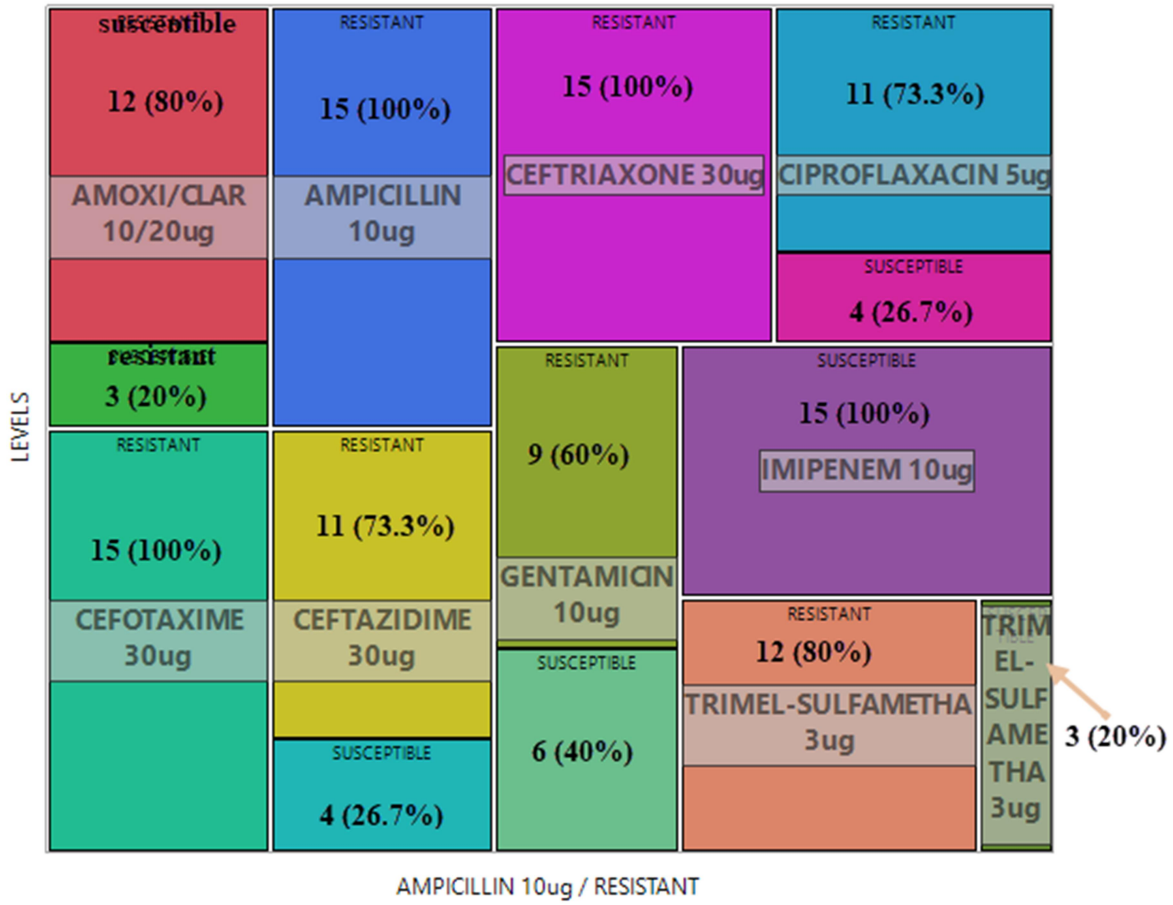

Figure 4: Antimicrobials susceptibility pattern for positive ESBL Producing-*K. pneumoniae*

The positive ESBL Producing-*K. pneumoniae* 12 (80%) were resistant to Amoxi/Clar (10/20ug) while, 3 (20%) were susceptible to Amoxi/Clar (10/20ug). Whereas 15 (100%) of the ESBL

Producing-*K. pneumoniae* were all resistant to Ampicillin (10ug). While 15 (100%) of the ESBL Producing-*K. pneumoniae* were all resistant to Ceftriaxone. Furthermore, the positive ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Ciproflaxacin (5ug) and 4 (26.7%) were susceptible to Ciproflaxacin (5ug). Additionally, 15 (100%) of the ESBL Producing-*K. pneumoniae* were all resistant to Cefotaxime (30ug). The positive ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Ceftazidime (30ug) while, 4 (26.7%) were susceptible to Ceftazidime (30ug). Whereas positive ESBL Producing-*K. pneumoniae* 9 (60%) were resistant to Gentamicin (10ug) while, 6 (40%) were susceptible to Ceftazidime (30ug). Whereas 15 (100%) of the positive ESBL Producing-*K. pneumoniae* were all resistant to Imepenem (10ug). The positive ESBL Producing-*K. pneumoniae* 12 (80%) were resistant to Trimel-Sulfamethan (3ug) while, 3 (20%) were susceptible to Trimel-Sulfamethan (3ug).

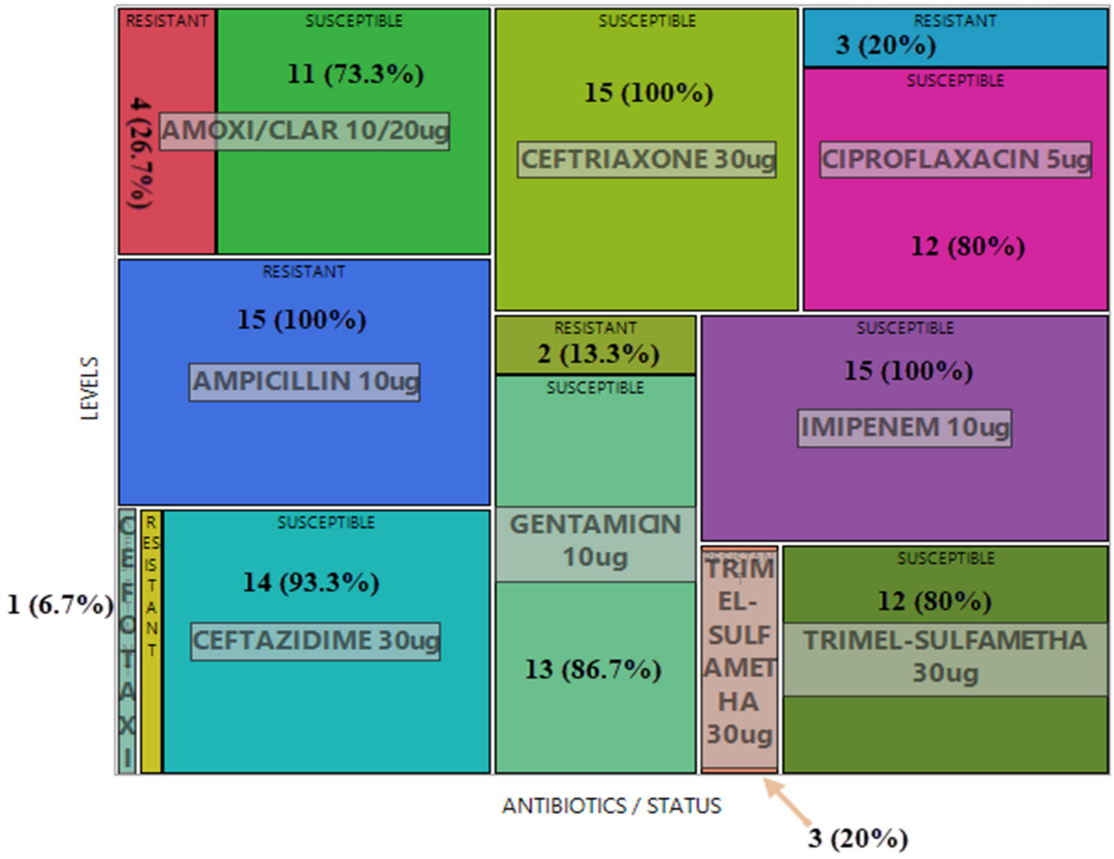

Figure 5: Antimicrobials susceptibility pattern for negative ESBL Producing-*K. pneumoniae*

The negative ESBL Producing-*K. pneumoniae* 11 (73.3%) were resistant to Amoxi/Clar (10/20ug) while, 4 (26.7%) were susceptible to Amoxi/Clar (10/20ug). Whereas 15 (100%) of the negative ESBL Producing-*K. pneumoniae* were all resistant to Ceftriaxone (30ug). While 3 (20%) of the negative ESBL Producing-*K. pneumoniae* were resistant to Ciproflaxacin (5ug) and susceptible to 12 (80%) of Ciproflaxacin (5ug). Furthermore, the negative ESBL Producing-*K. pneumoniae* 15 (100%) were all resistant to Ampicillin (10ug). Additionally, 2 (13.3%) of the negative ESBL Producing-*K. pneumoniae* were resistant to Gentamicin (10ug) and susceptible to 13 (86.7%). The negative ESBL Producing-*K. pneumoniae* 15 (100%) were all resistant to Imepenem (10ug). The negative ESBL Producing-*K. pneumoniae* 1 (6.7%) were resistant to Ceftazidime (30ug) while, 14 (93.3%) were susceptible to Ceftazidime (30ug). Whereas the negative ESBL Producing-*K .pneumoniae* 3(20%) were resistant to Trimel-Sulfamethan (30ug) while, 12 (80%) were susceptible to Trimel-Sulfamethan (30ug).

Discussion

Klebsiella pneumoniae is one of the most momentous multidrug-resistant (MDR) opportunistic Gram-negative bacteria. It is linked to dissimilar diseases which can cause high mortality and morbidity due to nosocomial and non-hospital acquired infections. In the current study, 65 (29.55%) and 57 (25.51%) were positive for the lactose fermenting organisms from both hospitals which were linked to urinary tract infections. These findings were in accord with the study of conducted in various countries (Yusha'u et al., 2010; Yahaya et al., 2016; Ahmed et al., 2017; Mengistu et al., 2018). Our findings also revealed that there was no significant associations between the hospitals and the infective agents.

Similarly, the current study revealed 19 (29.2%) and 11 (19.3%) from SSHM and USUMH respectively for *K. pneumoniae* from positive lactose fermenting organisms. These findings were similar to the findings divulged by Yahaya et al., (2016) in their study on the characterization of Klebsiella species.

Furthermore, result obtained from the double disc synergy method for the confirmation of ESBL producing- *K. pneumoniae* using Ceftazidime, Cefotaxime and amoxicillin/clavulanic acid (20/10 µg) in the present study showed 11 (57.9%) and 4 (36.4%) from SSHM and USUMH respectively for *K. pneumoniae*. These findings were similar to the reports of (Yusha'u et al., 2010; Yahaya et al., 2016). However our findings were slightly higher than their findings this could be due to the differences in the hospital settings.

Additionally, in the current study the susceptibility patterns of antimicrobials showed varying degree of resistance and susceptibilities for positive and non ESBL samples. Thus, all the positive ESBL samples were resistant to all the antimicrobials with the exception of Amoxiclav and Imipenem. Furthermore, the non ESBL producing-*K. pneumoniae* were all susceptible to all

the antimicrobials except Ampicillin which was resistant. These findings were similar to the study conducted by Mengistu et al., (2018) that non ESBL producing-*K. pneumoniae* may be resistant to Ampicillin by other mechanisms.

Conclusion

Carbapem (imipenem) is the most effective antibiotic for infections with *K. pneumoniae* for both ESBL and non ESBL producing-*K. pneumoniae.* This study revealed limited group of antibiotics that are ineffective for ESBL Producing *K. pneumoniae* and most of the antibiotics are effective for non ESBL producing *K. pneumoniae*. Our findings similarly discovered that there was no significant associations between the hospitals and the infective agents.

Recommendations

To ascertain the resistance and susceptibility of the antimicrobial agents against ESBL producing *K. pneumoniae*. There is need to use more antibiotic and antimicrobial agents. It is similarly important to identify the resistance genes associated with ESBL producing *K. pneumoniae* and the virulence genes associated with the ESBL genes.

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