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The Reliability of Using Vitek 2 Compact System to Detect Extended-Spectrum Beta-lactamase-producing Isolates in *Escherichia coli* and *Klebsiella pneumoniae* in Accra, Ghana

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ABSTRACT

Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases that are capable of hydrolysing β-lactams except carbapenems and cephamycins. The global increased prevalence of ESBL-producing bacteria creates an urgent need for laboratory diagnostic methods that will accurately and rapidly identify the presence of ESBL phenotypes in clinical isolates. The Vitek 2 System (bioMérieux, France) is a rapid automated microbiological system used for bacteria and yeast identification, antimicrobial susceptibility testing (AST), resistance mechanism detection and epidemiologic trending and reporting using its advanced expert system. This present work sought to determine the reliability of routinely using Vitek 2 System to accurately and rapidly detect ESBL-producing *E. coli* and *K. pneumoniae* in Accra. The ESBL phenotypes for 400 *E. coli and K. pneumoniae* isolates were determined using the Vitek 2 system and combined disc synergy method. The results were used to determine the sensitivity, specificity, negative predictive value and positive predictive value of the Vitek 2 ESBL test through comparative analysis with the combined disk synergy method which is the reference method recommended by CLSI. The findings of this work indicated that the sensitivity, specificity, positive predictive value and negative predictive value of Vitek 2 system was 98.5%, 98.9%, 99% and 98.5% respectively. Consequently, Vitek 2 system is a reliable semi-automated microbiology system which may be used for routine, accurate and rapid detection of ESBL strains in health facilities in Accra, Ghana.

Keywords: Vitek 2 Compact System, Extended spectrum beta-lactamase, bioMérieux, *E. coli* and *K. pneumonia*

1.0 Introduction

Extended-spectrum beta-lactamases (ESBLs) are plasmid-mediated beta-lactamases that are capable of hydrolysing β-lactams except carbapenems and cephamycins. They are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. They have been found in the *Enterobacteriaceae* and other Gram-negative bacilli (Paterson and Bonomo, 2005). Kesah and Odugbemi (2002) reported more than 40% ESBL production among *Enterobacteriaceae* isolates in Lagos, Nigeria. In 2006, Olysegun and others (2006) also observed 50% ESBL production rate in *K. pneumoniae* isolates studied from Northwestern Nigeria. In Ghana, Adu-Sarkodie (2010) reported that EBSL has been isolated from 50.3% *Klebsiella* and 49.7% *E. coli* in Komfo Anokye Teaching Hospital, Kumasi. Outbreaks of infection with ESBL-producing organisms have been reported from virtually every European country (Hanberger *et al*., 1999). In some parts of Asia, the percentage of ESBL production in *E. coli* and *K. pneumoniae* varies from 4.8% in Korea (Pai *et al*., 1999) to 8.5% in Taiwan (Yan *et al*., 2000) and up to 12% in Hong Kong (Ho *et al*., 2005). ESBLs have been found in 30 to 60% of klebsiellae from intensive care units in Brazil, Colombia *and* Venezuela (Otman *et al*., 2002). The global increased prevalence of ESBL-producing bacteria creates an urgent need for laboratory diagnostic methods that will accurately and rapidly identify the presence of ESBL phenotypes in clinical isolates. Routine ESBL detection is highly recommended because some ESBL-producing organisms appeared susceptible to cephalosporins *in vitro* using conventional breakpoints but ineffective *in vivo*. A failure to detect ESBLs and subsequent treatment with oxyimino-cephalosporins are associated with a higher risk of therapy failure (Paterson *et al*., 2001). Other reports also indicate higher mortality rates (Kim *et al*., 2002). The Clinical and Laboratories Standard Institute recommends a two-step phenotypic approach (CLSI, 2006), which involves screening for reduced susceptibility to more than one of the indicator antimicrobials (cefotaxime, ceftazidime, cefpodoxime, ceftriaxone, and aztreonam). After the ESBL screening test, the CLSI recommends the use of cefotaxime (30µg) or ceftazidime

disks (30µg) with clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs in *Klebsiella* species and *E. coli*. The CLSI recommends that the disk tests should be performed with confluent growth on Mueller-Hinton agar. A difference of ≥5mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production (CLSI, 2006). The combined disk synergy method is accepted as a reference method for confirming ESBL-producing organism according to CLSI (CLSI, 2006). The Vitek 2 System (bioMérieux, France) is a rapid automated microbiological system used for bacteria and yeast identification, antimicrobial susceptibility testing (AST), resistance mechanism detection and epidemiologic trending and reporting. It analyses MIC patterns and detects bacterial resistance mechanisms and phenotypes for most organisms tested using its advanced expert system. Vitek 2 ESBL test is reported to serve as a phenotypic confirmatory tool for rapid detection of a positive or negative ESBL producing strain which is based on simultaneous assessment of the inhibitory effects of cefotaxime, ceftazidime and cefepime, alone and in the presence of clavulanic acid (Teresa et al., 2006). This present work seeks to determine the reliability of routinely using Vitek 2 System to accurately and rapidly detect ESBL-producing *E. coli* and *K. pneumoniae* in Accra by determining the sensitivity, specificity, negative predictive value and positive predictive value of the Vitek 2 ESBL test through comparative analysis with the combined disk synergy method.

2.0 Materials and Methods

2.1 Materials

Glycerol broth, blood agar and MacConkey agar were prepared according to manufacturers' guidelines. MAST ID™ ESβL Detection Discs (Mast Group, UK) were used for ESBL screening and confirmation according to CLSI standards. Vitek 2 Compact System (bioMérieux, Marcy I'Etoile, France) was also used to detect ESBL producers based on simultaneous assessment of the inhibitory effects of cefotaxime, ceftazidime and cefepime, alone and in the presence of clavulanic acid.

2.2 Sample Size

A sample size of 400 *K. pneumoniae* and *E. coli* were collected from the Central Laboratory of the Korle Bu Teaching Hospital (KBTH) and Advent Clinical Laboratories; both in the Accra Metropolis, Ghana. This corresponds with the standard techniques used to calculate the minimum sample size based on the expected prevalence and using appropriate levels of precision at 95% confidence level.

2.3 Inclusion Criteria

Non-duplicate pure cultures of *K. pneumoniae* and *E. coli*.

2.4 Exclusion Criteria

All isolates not confirmed as *K. pneumoniae and E. coli*.

2.5 Identification of Bacterial Isolates and Determination of ESBL phenotypes using Vitek 2 System

The lactose fermenting isolates were sub-cultured on blood and MacConkey agar and incubated at 35°C for 24 hours. *K. pneumoniae* and *E. coli* were identified based on their Gram stain reaction and biochemical reaction characteristics using Vitek 2 system. Sterile test tubes (ID and AST test tubes) used to prepare inoculums were filled with 3ml of 0.45% saline water and placed in a cassette. The identification (ID) test tube was used to prepare inoculum from the pure colonies and mixed thoroughly using a vortex until a suspension of 0.5 – 0.63 McFarland was formed. The McFarland was determined using Densichek (bioMérieux, France). A volume of 45µl of the inoculum from the ID test tube was pipetted into the antibiotic susceptibility testing (AST) test tube and mixed thoroughly. The Gram negative (GN) ID test cards and AST test cards were inserted in the respective test tubes and loaded into the Vitek instrument. While in the Vitek instrument, the cards were filled, sealed and incubated in the Vitek 2 system incubator until results were generated by the expert advanced system of the Vitek 2 system for the type of organism and ESBL phenotype.

2.6 Detection of ESBL Phenotype using Combined Disc Synergy Method

MAST ID™ ESβL Detection Discs (Mast Group, UK) were used to screen and confirm the ESBL phenotypes. The MAST ID™ ESβL Detection Discs comprise of cefpodoxime 30µg disks, cefpodoxime 30µg + clavulanic

acid 10µg disks; ceftazidime 30µg disks, ceftazidime 30µg + clavulanic acid 10µg disks and cefotaxime 30µg disks, cefotaxime 30µg + clavulanic acid 10µg disks. Using a pure culture of the test organism, a suspension in distilled water equivalent in density to a McFarland 0.5 opacity standard was prepared. Using a sterile swab, the suspension was spread uniformly across the surface of Mueller-Hinton agar plate. Using a sterile forceps, one of each MAST ID^{TM} ESβL Detection Discs was placed onto the inoculated medium ensuring that they were evenly spaced. The plates were incubated aerobically at 35-37°C for 18 – 20 hours. The diameter of any zones of inhibition that were observed were measured and recorded. The zone of inhibition for the cefpodoxime, ceftazidime and cefotaxime was compared to that of the cefpodoxime, ceftazidime and cefotaxime plus clavulanic acid combination disks. An increase in zone diameter of ≥5mm in the presence of clavulanic acid from any or all of the sets of MAST ID^{TM} ESβL Detection Discs indicates the presence of ESBL in the test organism.

2.7 Statistical Analyses

In comparison with results of the Combination Disk Synergy Method, the sensitivity of Vitek 2 ESBL test was calculated as the number of true positive ESBL divided by the sum of true positive and false negative ESBL X 100. The specificity of Vitek 2 ESBL test was calculated as the number of true negative ESBL divided by sum of true negative and false positive ESBL X 100. The positive predictive value was calculated as number of true positives divided by the sum of true positive and false positives X 100. The negative predictive value was calculated as number of true negatives divided by sum of true negatives and false negatives X 100. The data from the work was collated and statistically analysed using one-way analysis of variance (ANOVA). Results were considered significant if $p<0.05$

3.0 Results

3.1 Bacterial Isolates

Of the 400 bacterial isolates collected, 175 were *K. pneumoniae* and 225 were *E. coli* as shown by table 1. The bacterial isolates were produced from various clinical specimens submitted to the Korle Bu Central Laboratory and Advent Clinical Laboratories all in Accra.

Table 1: Number of Bacterial Isolates

K. pneumoniae	*E. coli*	Total
175 (43.7%)	225 (56.3%)	400 (100%)

3.2 ESBL Producing Phenotypes

Two ESBL-producing detection methods (Vitek 2 ESBL test and combined disc synergy method) were used in determining the ESBL phenotypes of the bacterial isolates. The Vitek 2 ESBL test indicated that of the 175 *K. pneumoniae* isolates, 129 (73.7%) were ESBL producers and 73 (32.4%) of the 225 *E. coli* isolates were ESBL-producing phenotypes. The total ESBL producing isolates detected by the Vitek 2 Compact System was 202 representing 50.5% of the 400 bacterial isolates as demonstrated in table 2. The combined disc synergy method (CDM) detected 203 (50.8%) of ESBL producers among the 400 total bacterial isolates of which 130 (74.3%) of the 175 *K. pneumoniae* and 73 (32.4%) of the 225 *E. coli* isolates were ESBL producers as shown in table 2. There was no significant difference ($p\geq0.05$) between the ESBL phenotypes detected by the combined disc synergy method and the Vitek 2 ESBL test in both the *K. pneumoniae* and *E. coli* isolates.

Table 2: Occurrence of ESBL-producing Phenotypes

	Number (%)		
ESBL Detection Method	*K. pneumoniae* n=175	*E. coli* n=225	All Isolates n=400
CDM	130(74.3)	73(32.4)	203(50.8)
Vitek 2 System	129(73.7)	73(32.4)	202(50.5)

CDM: Combined Disk Synergy Method

3.3 Non-ESBL Producing Phenotypes

The Vitek 2 ESBL test indicated that of the 175 *K. pneumoniae* isolates, 46 (26.3%) were non-ESBL producers and 152 (67.5%) of the 225 *E. coli* isolates were non-ESBL producing organisms. The total non-ESBL producing isolates detected by the Vitek 2 Compact System was 198 representing 49.5% of the 400 bacterial isolates as demonstrated in table 3. The combined disc synergy method detected 197 (49.3%) of non-ESBL producers among the 400 total bacterial isolates of which 45 (25.7%) were *K. pneumoniae* and 152 (67.5%) were *E. coli* isolates as shown in table 3.

Table 3: Distribution of Non-ESBL-Producing Phenotypes

	Number (%)		
ESBL Detection Method	*K. pneumoniae* n=175	*E. coli* n=225	All Isolates n=400
CDM	45(25.7)	152(67.5)	197(49.3)
Vitek 2 Compact	46(26.3)	152(67.5)	198(49.5)

CDM: Combined Disk Synergy Method

3.4 Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Vitek 2 Compact System

The reliability of Vitek 2 Compact System as an ESBL detection system was verified in comparison with the combined disk synergy method which is recommended by CLSI. As indicated in table 4, the true positive, true negative, false positive and false negative ESBL strains among the 400 bacterial isolates as detected by Vitek 2 ESBL test were 200, 195, 2 and 3 respectively. Consequently, the sensitivity, specificity, positive predictive value and negative predictive value of Vitek 2 Compact System among the 400 bacterial isolates was 98.5%, 98.9%, 99.0% and 98.5% respectively as shown in figure 1.

Table 4: Reliability of Vitek 2 Compact as ESBL Detection System

Parameters	*K. pneumoniae* n=175	*E. coli* n=225	All Isolates n=400
True Positive	128	72	200
True Negative	44	151	195
False Positive	1	1	2
False Negative	2	1	3

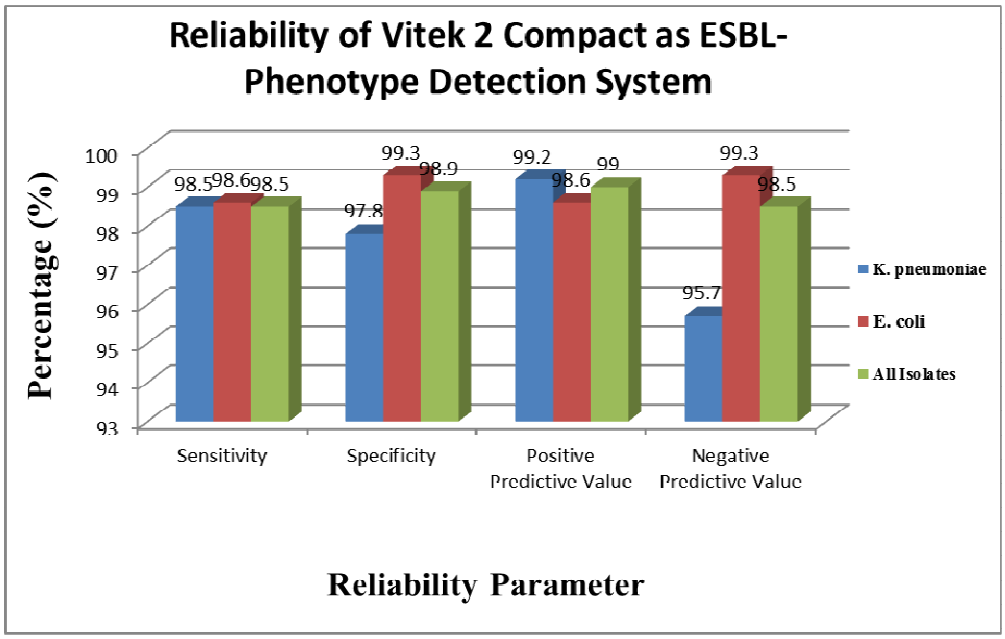

Figure 1: Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Vitek 2 Compact System

4.0 Discussion

VITEK 2 compact system (bioMérieux, Marcy I'Etoile, France) is a semi-automated bacterial identification and susceptibility testing system enabling rapid determination of MICs by analysis of bacteria growth kinetics with antimicrobials in sealed test cards and resistant mechanisms. This study aimed at establishing the reliability of VITEK 2 compact system to detect ESBLs in clinical isolates of *K. pneumoniae* and *E. coli* in comparative analysis with combined disk method. In a comparative study with CLSI method of detecting ESBL, Sorlozano and colleagues (2005) observed that the sensitivity (100%), specificity (99.3–100%), and predictive values of the disk approximation, Etest and VITEK 2 methods were similar. This outcome is comparable to the sensitivity (98.5%), specificity (98.9%), positive predictive value (99%) and negative predictive value (98.5%) of Vitek 2 system as observed in this present work. The sensitivity and specificity values obtained were somewhat better than those reported by Leverstein-van Hall *et al*. (2002) (100% sensitivity, 87% specificity), Sanders *et al*., (2000) (91% sensitivity), and Livermore *et al*. (2002) (93% sensitivity), although these studies evaluated ESBL positive strains belonging to various species other than *K. pneumoniae* and *E. coli*. Nevertheless, they concluded that VITEK-2 compact showed an acceptable reliability to detect ESBL-producing *K. pneumoniae* and *E. coli*.

Wiegand and colleagues (2007) compared the ability of three commercially available semi-automated microbiology identification and susceptibility testing systems [Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD), the VITEK 2 System (bioMérieux, Marcy l'Etoile, France) and the MicroScan WalkAway-96 System (Dade Behring, Inc., West Sacramento, CA)] to detect ESBL production in *Enterobacteriaceae* using routine testing panels. The bacterial isolates investigated included *Escherichia coli,*

Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Serratia marcescens, Proteus mirabilis, Proteus vulgaris and Morganella morganii. Of these 147 isolates used, 85 were identified as ESBL producers by the reference method. The system with the highest sensitivity for the detection of ESBLs was the Phoenix (99%), followed by the VITEK 2 (86%) and the MicroScan (84%); however, specificity was more variable, ranging from 52% (Phoenix) to 78% (VITEK 2). The performance of the semi-automated systems differed widely with the species investigated (Wiegand *et al*., 2007). This outcome contradicts the sensitivity and specificity values observed in this present work.

However, a work published by Teresa and colleagues (2006) which agreed with the outcome of this present work suggested that Vitek 2 system appears to be a rapid and reliable tool for routine identification of ESBL-producing isolates of *Enterobacteriaceae*. They examined a total of 1,129 clinically relevant *Enterobacteriaceae* isolates for ESBL producing using Vitek 2 system and molecular method and the results concluded that the VITEK 2 ESBL test system was concordant with that of the comparison method (molecular identification of beta-lactamase genes) for 1,121 (99.3%) of the 1,129 isolates evaluated. ESBL production was correctly detected in 306 of the 312 ESBL-producing organisms (sensitivity, 98.1%; positive predictive value, 99.3%). False-positive results emerged for 2 of the 817 ESBL-negative isolates (specificity, 99.7%; negative predictive value, 99.3%). VITEK 2 ESBL testing took 6 to 13 h (median, 7.5 h; mean ± SD, 8.2 ± 2.39 h) (Teresa *et al*., 2006).

5.0 Conclusion

The findings of this work indicated that the sensitivity, specificity, positive predictive value and negative predictive value of Vitek 2 system was 98.5%, 98.9%, 99% and 98.5% respectively in comparison with the combined disc synergy method which is the reference method as recommended by CSLI. Consequently, Vitek 2 sytem is a reliable semi-automated microbiology system which may be used for routine, accurate and rapid detection of ESBL strains in health facilities in our settings.

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