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COMPARATIVE ANALYSIS OF THREE METHODS FOR SCREENING FOR COLISTIN RESISTANT *ESCHERICHIA COLI*

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ABSTRACT

The usage of colistin regarded as a drug of last resort has increased tremendously in recent years. This increase has been followed by an increase in the development of colistin resistant bacteria. Due the large size of colistin and its ability to adhere to plastic, the broth microdilution method using a special medium is the recommended testing method. Resource limited settings struggle with this method and employ alternate methods. This study therefore set out to determine colistin resistance in a group of Escherichia coli using three different methods comprised of colistin agar spot test (COL-AS), colistin drop test (COL-DT) and a disc diffusion method. A total of 51 Escherichia coli isolated from wound samples were screened for colistin resistance using the COL-AS, COL-DT and colistin disc diffusion methods. Results showed a combined resistance rate of 96.1% among test isolates. Actual resistance rates varied between testing methods giving values of ranging from 37.3%, 66.0% and 88.2% for COL-DT, colistin disc diffusion methods and COL-A respectively. An assessment of test performance showed categorical agreement values and very major error values of 57.1%/36.7% for COL-DT and 63.3%/8.2% for COL-A. Results of this study show a high-level occurrence of colistin resistance among clinical Escherichia coli isolates. Furthermore, it demonstrates the superiority of the colistin agar test to the colistin drop test. It also points at a need to use higher concentrations of colistin in the screening tests.

Keywords: Colistin, screening tests, COL-A, COL-DT

INTRODUCTION

Colistin is regarded as a drug of last resort, which means the drug considered for patients' use when all other drugs fail. Colistin is an antibiotic belonging to the polymyxin drug family. This class of drug was discovered as far back as 1949 and is widely used for agricultural purposes (Andrade et al.,2020). Although initially used in human therapy, the use of colistin was discontinued due to serious nephrotoxicity and neurotoxicity (Olaitan andLi 2016; El-Sayed et al.,2020). In more recent years, this drug has however been reintroduced for human therapy due to the increasing menace of drug resistance and a need for antibiotics effective against key bacteria (Coetzee, 2016). Colistin is only effective against Gram negative bacteria with Gram positive bacteria exhibiting intrinsic resistance to this drug. This drug is bacteriocidal and acts in several ways. One of these mechanisms, involves extracting divalent cations from the negatively charged

phosphate groups of cell membrane of the bacteria resulting in an unstable membrane causing a release of intracellular components and hence lysis and death (Bolla et al.,2011; Petrosilloet al.,2019; Andrade et al.,2020). One key use for colistin is against "super bugs" such as Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* which exhibit resistance to basically most commonly used antibiotics (Azzopardi et al.,2013).

In the yearssince the re-introduction of colistin into clinical practice in the 2000s there has been an increase in the development of colistin resistant bacteria with an increase from 2.9% to 12.9% reported in *Klebsiella pneumoniae*studied between 2015 and 2019 and 2020 respectively(Shahzad et al., 2023). With colistin being a drug of last resort, these bacteria were theoretically untreatable (Wallace, 2011). Colistin resistance has been reported worldwide (Ngbedeet al.,2021). In recent years following the discovery of a plasmid mediated form of colistin resistance in addition to the traditionally known resistance brought about by chromosomal mutations, interest in colistin resistance increased (Skov and Monnet, 2016, Anyanwu et al.,2021). A recent review observed an increase in occurrence of colistin resistant bacteria between 1973 and 2019 (Yacouba andOlowo-Okere, 2020). One study reviewing colistin resistance in 2022, noted that the highest colistin resistance pooled prevalence was recorded in isolates studied in 2020 and beyond was 12.90% (4/31) globally (Uzairueet al.,2022).

Studies exploring colistin resistance in Nigeria were previously lacking, but this has changed in more recent years. A PubMed search on colistin and Nigeria for 2019 showed 32 articles but by 2023, 69 articles had been published (PubMed search 3rd February 2024). Because of the nature of the colistin drug particularly its large size leading to poor diffusion ability and its ability to adhere to plastic, the recommended method for testing for colistin resistance is more complicated than the average resistance testing method (Humphries, 2015; Jouy et al.,2017). This method involves microdilution and the use of a special media. It has been observed that resource limited studies might generally struggle with deploying this method (Pasteran et al.,2020). One study described alternative methods which might be more appropriate for resource limited settings and showed the methods to give comparable results to the EUCAST standard (Jouy et al.,2017). This study reported categorical agreements above 95% with broth microdilution (BMD) for colistin agar spot test (COL-AS) and colistin drop test (COL-DT).

Studies from Nigeria have used methods covering colistin drop test, agar test, disc diffusion, e-test, agar diffusion, and combined disc test (Omoruyi et al.,2023; Iroha et al.,2023). A number of studies in Nigeria exploring colistin resistance make use of only colistin agar testing method. As this method simply involves the culture of test isolates on media containing 2 µg/ml of colistin. Therefore, this study compares colistin resistance in a group of *Escherichia coli* using three different methods that include: COL-AS, COL-DT and disc diffusion method.

MATERIALS AND METHODS

Sample Collection and characterisation

Test isolates comprised of fifty-one *Escherichia coli* isolated from wound samples at the University of Port Harcourt Teaching Hospital (UPTH). To confirm isolate identities, bacteria were first sub-cultured to Eosin methylene blue agar to check for characteristic colonies giving a green metallic sheen. These characteristic colonies were then purified on nutrient agar and the isolate identities confirmed using standard biochemical tests which include oxidase, coagulase, catalase, indole, methyl red, Voges Proskauer, triple sugar iron fermentation, starch hydrolysis, urease,

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citrate, motility and sugar fermentation tests (Cheesbrough, 2006).

Testing for Colistin Resistance

The colistin susceptibility test was carried out using three different methods that include: colistin drop test (COL-DT) as described by Jouy et al., (2017) and modified by Pasteran et al.,(2021), colistin agar (COL-A) method described by Ali et al.,(2021) and disc diffusion method using 10 µg colistin sulphate (Oxoid, UK).

Colistin Drop Test (COL-DT)

The colistin drop test was carried out using colistimethate sodium. A single 2 µl drop of 0.001 mg/ml was placed on a Mueller Hinton Agar plate previously swabbed with inoculum concentration corresponding to 0.5 McFarland standard of the test isolate. Following 15 min pre-incubation at room temperature, the plate was incubated at 35°C for 18 hours. After incubation, a careful observation with transmitted light in the existence or non-existence of an inhibitory zone was made. Halos were recorded for standardization's sake. An isolate was classified as colistin sensitive if any zone of inhibition was seen (zones for susceptible isolates are around >5 mm) (Jouy et al., 2017). An isolate was deemed to be colistin resistant if there was no halo surrounding the drop or colonies in the zone of inhibition, which are indicators of resistant subpopulations.

Colistin Agar (COL-A)

In this method, an inoculum corresponding to 0.5 McFarland standard was diluted 1:10 and streaked on a Mueller Hinton Agar plate containing 2µg/ml of colistin. Set up was then incubated for 18 hours at 37°C. Presence of growth was indicative of a colistin resistant isolate and absence of growth indicative of a colistin sensitive isolate.

Colistin sulfate disc diffusion method

Single antibiotic discs containing Colistin Sulfate 10 µg (Oxoid, UK) was placed on the surface of Mueller-Hinton agar inoculated with test isolates corresponding to 0.5 McFarland standard. The plates underwent incubation at 37ºC for 24hrs. The measured zone of inhibition was then interpreted using previously described reference values (Tan and Ng, 2006).

Data Analysis

Data generated was then analysed based on the method previously described (Pasteran et al.,2020), with a slight modification to determine the Categorical agreement (CA), Very major errors (VME) and Major errors (ME). Where categorical agreement assesses the rates isolates agree as sensitive or resistant compared to the standard, Very major errors points at isolates determined as resistant by the standard but called as sensitive by the screening tests, and Major errors, isolates sensitive by the standard but resistant by the screening tests.

RESULTS

Colistin resistance in Test Isolates

An analysis of colistin resistance in test isolates using the three methods gave a combined resistance rate of 96.1% (Figure 1). These rates, however, were quite varied based on test method with prevalence rates ranging from 37.3% to 88.2% (Figure 2).

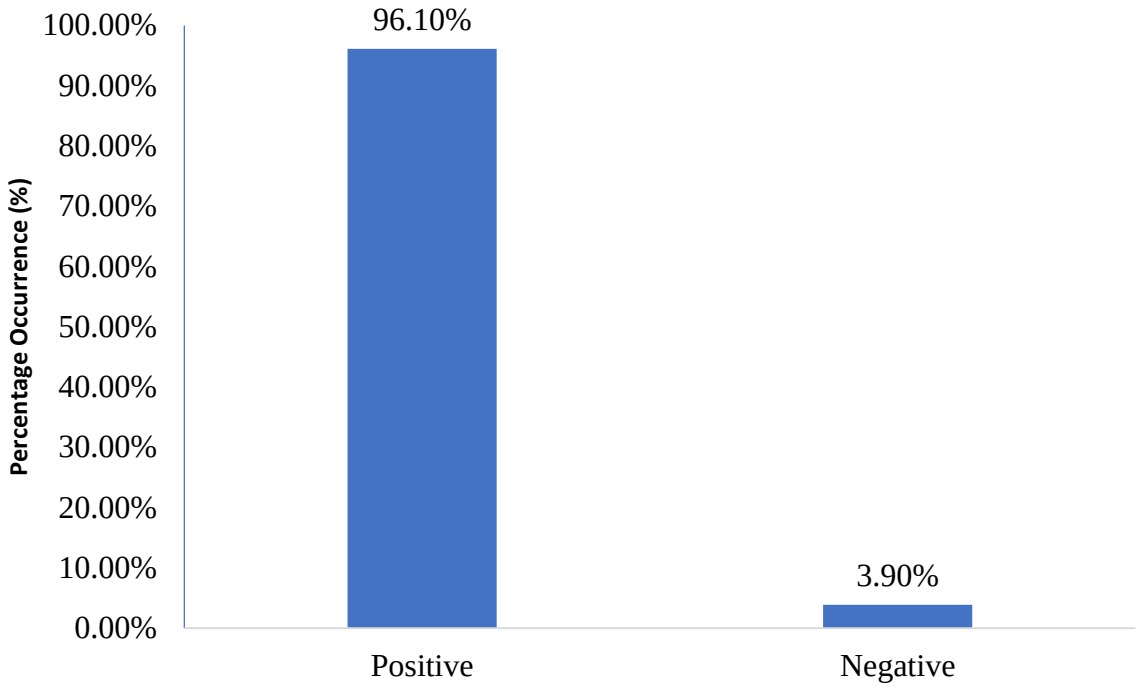

Figure 1: Overall occurrence of colistin resistance among test isolates

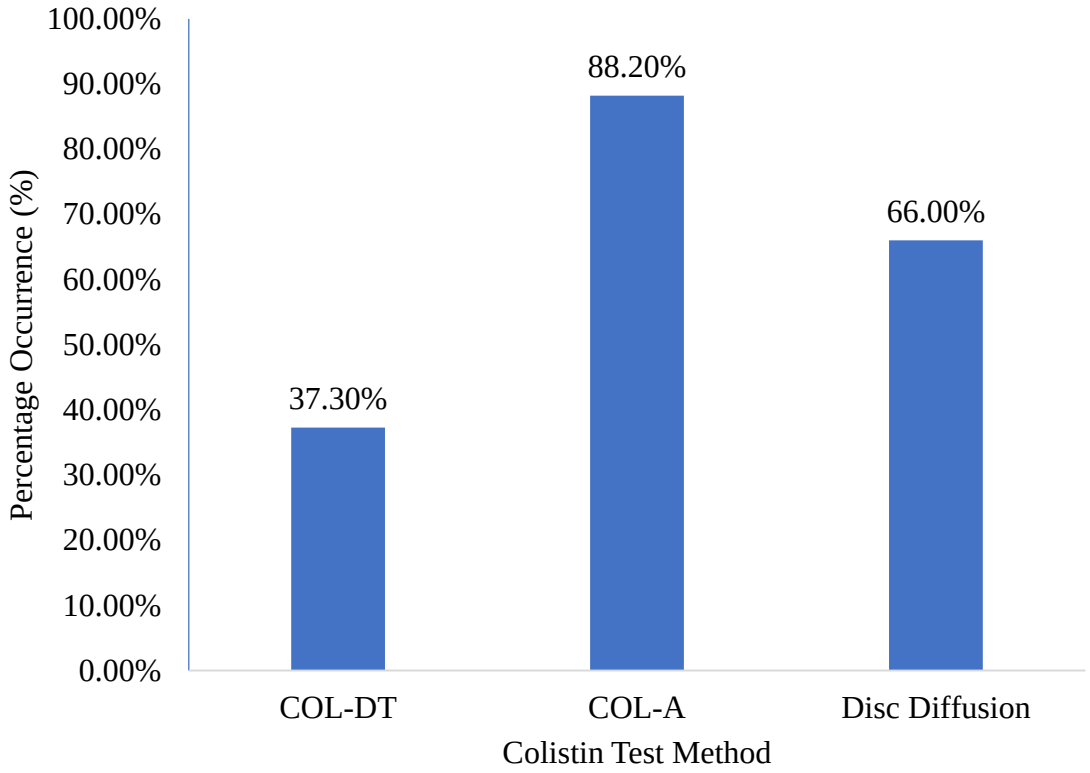

Figure 2: Comparison of test-based variation in detection of colistin resistance in *Escherichia coli*

Of the 49 isolates with a colistin positive result from any of the tests, 15 (30.6%) were positive with all three methods used, while 19 (38.8%) were positive with any 2 tests employed (Figure 3)

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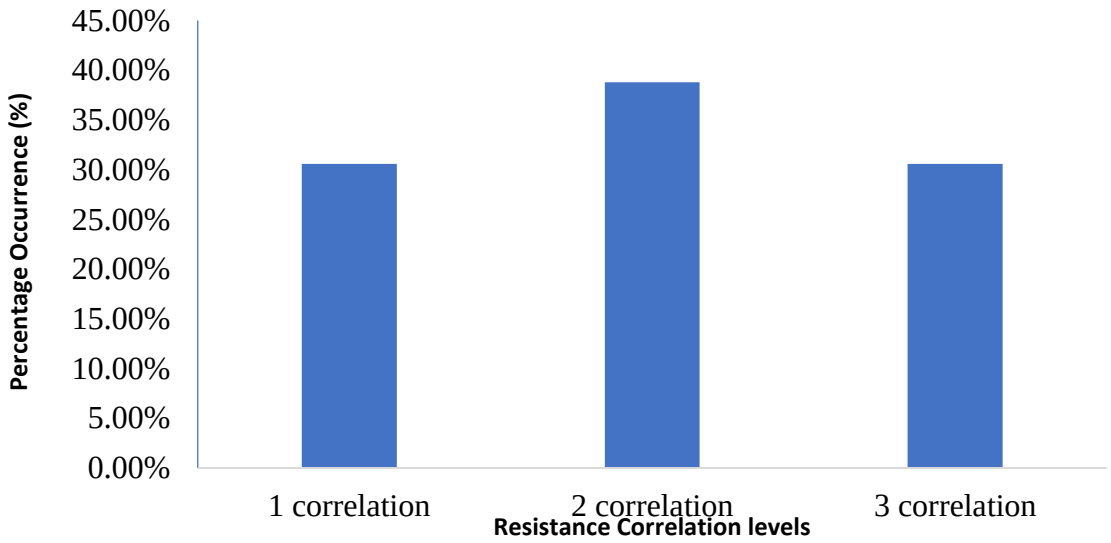

Figure 3: Rate of correlation of resistance between tests

An assessment of the performance of the different tests against a standard showed that the COL-A test performed better with respect to CA and VME values (Table 1). Both tests however had significantly low CA values and for COL-DT, a very high VME value (36.7%) was observed.

Table 1: Assessment of screening tests performance using DD as standard

	COL-DT	**COL-A**
Categorical agreement	57.1%	63.3%
Very major errors	36.7%	8.2%
Major errors	10.2%	32.7%

DISCUSSION

The assessment in this study for colistin resistance using the three methods noted rates ranging from 37.3% to 88.2% with a combined rate of 96.1%. This high rate is similar to that previously reported in 2022 by Uddin and colleagues who identified 88.3% colistin resistance in *E. coli* isolated from chicken (Uddin et al.,2022).These values were however significantly higher than most other studies reporting rates of up to 8% (Kaza et al.,2019; Omoruyi et al.,2023; Iroha et al.,2023; Abdullahi et al.,2022)This was perhaps a location based effect. Dadashi and colleagues in 2021, presenting a review on colistin resistance had noted that Nigeria had one of the highest levels of colistin resistance rates (38.36%) globally (Dadashi et al., 2021).

Due to the large size of the colistin antibiotic, susceptibility testing using diffusion-based methods are often found to be unreliable and, in some cases, unreliably give false susceptible results (Satin, 2019). On the other hand, a study carried out specifically on *Acinetobacter* sp. found that 10 isolates called resistant by disc diffusion were actually sensitive by both e-test and the broth microdilution method (Jeram et al.,2021). One previous study had, however, noted a high concordance (92%) between the disc diffusion tests and broth microdilution tests (Behera et al.,2010.). The Behera study suggests that agar dilution might be a suitable method as an initial screen. Soria-Segarra and colleagues in a 2022 paper showed that the colistin agar test had a high level of agreement with the broth microdilution(BMD) method with a 98% CA score noted and ME of 2.97% (Soria-Segarra et al.,2022). This study was carried out on Enterobacterales in general. This suggests a level of discordancy in the testing methods

and could point at interlaboratory variables arising from type of agar, errors in concentrations or pipetting variables.

In this study, a comparison of the three tests showed that the Colistin Agar test did better than the Colistin drop test. This superiority of the COL-A method over the COL-DT method has been previously reported (Gonzales - Escalante et al.,2020; Pasteran et al.,2020). A number of these studies showed variations in CA between the two methods ranging from 90.48% to 96.2% (AlHirakyet al.,2021; Pasteran et al.,2020). Despite the better results observed by the Colistin Agar test, it must be pertinent to know that none of the two methods would be considered suitable as they both failed to meet the >90% CA cutoff recommended for acceptance (Conceicao-Neto et al.,2020). One suggestion would be to increase the concentrations of colistin used for the screening. Although 2 µg/ml is widely used, it has been recommended that due to the higher margin for error at lower MICs, the use of higher concentrations during screening increases the chances of improved detection of colistin resistant isolates.

CONCLUSION

Results of this study show a high-level occurrence of colistin resistance among clinical *Escherichia coli* isolates. Furthermore, it demonstrates the superiority of the colistin agar test to the colistin drop test. And points at a need to use higher concentrations of colistin in the screening tests.

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