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Original Article

PREVALENCE OF ESBL-POSITIVE E. COLI ISOLATED FROM BLOOD AND PUS SAMPLES (JANUARY 2019 TO MARCH 2021) SPECIMENS AT MAKERERE UNIVERSITY CLINICAL MICROBIOLOGY LABORATORY. A CROSS-SECTIONAL STUDY.

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Abstract

Background

Production of extended-spectrum beta-lactamase enzymes is one of the common mechanisms of resistance of *Escherichia coli* to antibiotics, thus timely detection and reporting of this phenotype are very critical and crucial especially when it comes to bloodstream and wound infections caused by this phenotype of *E.coli*. Therefore, this study sought to determine the prevalence of ESBL-Positive *E.Coli* isolated from blood and pus samples.

Methodology

This study was a cross-sectional retrospective analysis of lab records for ESBL-positive *E. coli* isolated from blood and pus samples received between the periods of January 2019 to March 2021 at the Makerere University Clinical Microbiology Laboratory, purposive sampling technique was used to select only records for which ESBL positive *E.coli.*

Results

For Bloodstream infections: Out of the 91 samples positive for *E.coli* received by the lab between the periods of January 2019 to March 2021, 50.55% (46) were ESBL positive while 46.15% (42) were ESBL negative. 3.30% (3) of the samples were not screened for ESBL production. In the majority (46) 51% of blood cultures were positive for ESBL. *E.coli* and 42(46%) were ESBL negative *E.coli.* **For wound infections**: Out of 85 samples positive for E.coli received by the lab between the periods of January 2019 and March 2021, 44.71% (38) were ESBL positive while 55.29% (47) were ESBL negative. The majority 47 (55.29%) of pus swabs from wound infections were ESBL negative *E.coli* while 38(44.71%) were ESBL positive *E.coli.*

Conclusion

50.55% of the isolated *E.coli* from bloodstream infections were ESBL positive as compared to wound infections, the prevalence was 44.71%. This could pose a great risk to the management of *E.coli* infections.

Recommendation

The laboratory should play the role of guiding physicians and medical personnel on the need for infection control measures to prevent the spread of ESBL *E.coli* organisms.

Key words; *Makerere University, prevalence of ESBL-Positive* E.Coli, *Blood and pus samples.*
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Background of the study

Escherichia coli is a lactose fermenting gram-negative motile bacteria commonly occurring as normal flora in the gut, despite being a commensal microorganism, pathogenic strains like extraintestinal pathogenic *E. coli* (ExPEC) strains are commonly associated with wound and bloodstream infections (Leimbach et al., 2013). Globally *E.coli* is responsible for approximately 20% of all clinically significant isolates in blood cultures and *51.2*% of isolated pathogens resulting in deep wound infections and diseases like osteomyelitis. (Kumar et al., 2020; Trojan et al., 2016). A study in two tertiary hospitals in Eastern Uganda discovered that *Escherichia coli* was the most prevalent 33.9% 0fisolated bacteria in cultures. (Obakiroet al., 2021)

It's estimated that severe *E. coli* sepsis causes approximately 40,000 deaths per year (Sharma et al., 2011). Fatality rates for bacteremia are between 13% and 19% but may be much higher (up to 60%) in elderly persons with nosocomial infections. (Roubaud Baudron et al., 2014) Factors like: age very young or old age; underlying respiratory infections and ciprofloxacin non-susceptibility were associated with high mortality rates. (Trojan et al.,2016, Mora-Rillo et al., 2015)

Extraintestinal pathogenic *E.coli* (ExPEC) poses specific virulence factors (VFs) that play a role in enabling the bacterial cells to colonize the host, disseminate, and

survive in blood and various tissues causing bloodstream and wound infections. VFs are either encoded on the bacterial chromosome or plasmids; they include adhesion molecules, iron acquisition systems, host defense-subverting mechanisms, and toxin production (Daga et al., 2019). The emergence of extended-spectrum β-lactamase (ESBL) *E.coli* strains that can produce enzymes that make them resistant to penicillins and cephalosporins of the first, second, and third generations as well as aztreonam through hydrolysis of these antibiotics presents as a challenge in the management of *E.coli* isolated from the bloodstream and wound infections.

Currently, the prevalence of blood bloodstream and wound infections caused by ESBL-producing *E.coli* is estimated to be at 94.6 % and 60% respectively. (*JCDR - Drug Resistance, Multiple, Bacterial, Escherichia Coli Infections/Microbiology*,n.d.; Kibret &Abera,2011)

In a study done in Uganda to determine the prevalence of ESBL producers in cultures, 60% of the isolates were *Escherichia coli* isolates. Without early detection of ESBL-producing *E.coli* in the lab, treatment failure and disease complications may arise. (Kasango et al., 2018)

Therefore, timely and accurate detection of extended-spectrum beta-lactamase-producing *Escherichia coli* in blood and pus cultures is crucial in in-patient management and is dependent on quality control in all phases of the lab to prevent errors. However, the increase in the prevalence of diagnostic errors presents a challenge to the accurate and timely detection of extended-spectrum beta-lactamase *Escherichia coli-positive* samples. These Diagnostic errors could occur in either the pre-analytical, analytical, or post-analytical phase of lab diagnosis and may result in misdiagnosis, inappropriate therapeutic interventions, unnecessary investigations, diagnostic delays, mix-up of patient results, prolonged hospital stay, delays in reporting, unnecessary re-draws/re-tests, decreased customer satisfaction, increased costs, incorrect diagnosis, injury and occasionally death. (Green, 2013; State, 2015) The highest error rates were found in Blood (25.57 %), and wound cultures (12.06%)(*(PDF) Analysis on the Errors in the Pre-Analytical Process in a Clinical Microbiology Laboratory/ BirMikrobiyoloji Laboratuvarındaki Preanalitik Sürçteki Hataların Analizi*, n.d.; Nichols, n.d. Valenstein, et al., n.d.). Hence, this study sought to determine the prevalence of ESBL-Positive *E.Coli* isolated from blood and pus samples.

Methodology

Study Design

This study was a cross-sectional retrospective analysis of lab records for ESBL-positive *E. coli* isolated from blood and pus samples received between the periods of January 2019 to March 2021 at the Makerere University Clinical Microbiology Laboratory.

Study Area

The study was conducted at Makerere Clinical Microbiology Laboratory using lab records for ESBL-positive E. *coli* isolated from pus and blood samples. The Microbiology Clinical laboratory is found at the College of Health Sciences, Makerere University. It's a level 2 biosafety laboratory, accredited by the College of American Pathologists (CAP number 7225593) under the Department of Medical Microbiology.

Study Population

The study population included all records of ESBL-positive *E.coli* isolated from blood and pus cultures obtained from patient test results from the period of January 2019 to March 2021 at the Makerere Clinical Microbiology Laboratory, College of Health Sciences, Makerere University.

Study selection criteria

Inclusion criteria

All records of ESBL-positive E. coli isolated from blood and pus cultures collected between the periods of January 2019 to March 2021 at the Makerere Clinical Microbiology Laboratory were included in this study.

Exclusion criteria

Records of other *E.coli*phenotypes isolated from blood and pus cultures were excluded from this study.

Sample Size Determination

The sample size was calculated using the Kish-Leslie formula (1965) below

N=

N=

N= 148 samples

Where, N = the desired sample size.

Z = the standard normal deviation 1.96, at a 95% confidence interval.

P = 44.4% prevalence of diagnostic lab errors as identified by a study done to determine errors in sample processing in the lab (Carraro & Plebani, 2007).

Q = 1-P

d2 = maximum error the investigator is willing to allow, (8%).

Study variables

Dependent variables

This variable was the timely and accurate reporting (turnaround time) of samples positive for ESBL *E.coli* isolated from blood and pus cultures.

Independent variables

The independent variables included;

Prevalence of ESBL-positive *E. coli*.

Pre-analytical errors like missing information on the lab request form e.g. missing age, name, sex, lab identification number, specimen type, test, and initials of recipient.

Analytical errors due to non-conformity with standard operating procedures for processing blood and pus samples e.g. missing gram stain, subculture, biochemical test, antimicrobial susceptibility test results, and initials of lab personnel who carried out the test

Post analytical errors like wrong data entry and increased turnaround time of results.
Other factors beyond control e.g., electricity, water, reagents shortages.

Sampling technique

A purposive sampling technique was used to select only records for which ESBL positive *E.coli*were reported by the lab between the period January 2019- March 2021.

Data collection tools

A checklist was used to collect data on lab errors occurring at the different stages of the lab cycle from sources like the sample reception, blood, and pus culture books.
For the preanalytical phase, data was collected using the sample reception book and the laboratory request forms to identify any errors that occurred like missing age, name, sex, lab identification number, specimen type, test, and initials of the recipient.
For the analytical phase, data was collected from the blood culture book and Pus swab book of the Makerere Clinical Microbiology Laboratory and used to identify any errors that occurred due to failure to follow standard operating procedures while processing the samples like; missing gram stain, subculture, biochemical test, antimicrobial susceptibility test results and initials of lab personnel who carried out the test.
The blood culture book and Pus swab book were used to monitor turnaround time which was calculated as the difference in time between when the sample was received at the lab and the time the results were reported or dispatched.
The prevalence of ESBL-positive *E.coli* was calculated using results recorded in the blood and pus culture books of the clinical microbiology laboratory.

Data Analysis and presentation

The data collected was checked for correctness and completeness. The data was then entered into a data capture tool (EPIDATA), validated, and exported to STATA version 13 for analysis.

This statistical analysis aimed at establishing the prevalence of ESBL-positive *E.coli* and determining the effect of laboratory errors on the accurate and timely reporting of bloodstream and wound infections caused by extended-spectrum beta-lactamase-positive *Escherichia coli* over the stated study period.
Quantitative data was then presented in the form of pie charts, tables, graphs, and written information.

Quality control

Data was extracted by two people to ensure accuracy and consensus, this made certain that no details were left eliminated or repeated.

Ethical consideration.

The study got ethical clearance from the higher degree and graduate research ethics committee (HDREC) of the School of Biomedical Sciences, Makerere University College of Health Sciences.
Permission to collect data was sought from the laboratory director through the Head of the department of medical microbiology and the laboratory Manager of the clinical microbiology laboratory to carry out a research study within their premise.
A waiver of consent was applied for from the laboratory management. This research only commenced after approval by the Institutional Review Board.
The patient details were kept with utmost confidentiality and were only accessed by study investigators who returned the documents to the laboratory immediately after use.
Data entries and results were identified by unique codes generated in the laboratory rather than patient names.

Results

Effect of Laboratory Errors on the Accurate and timely reporting of Bloodstream Infections Caused by extended-spectrum beta-lactamase *Escherichia coli*

Prevalence of ESBL-positive *E.coli* isolated from blood cultures.

Out of 91 samples positive for E.coli received by the lab between the periods of January 2019 and March 2021, 50.55% (46) were ESBL positive while 46.15% (42) were ESBL negative. 3.30% (3) of the samples were not screened for ESBL production.

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Figure 1: Prevalence of ESBL positive E.coli in bloodstream infections, (N=91)

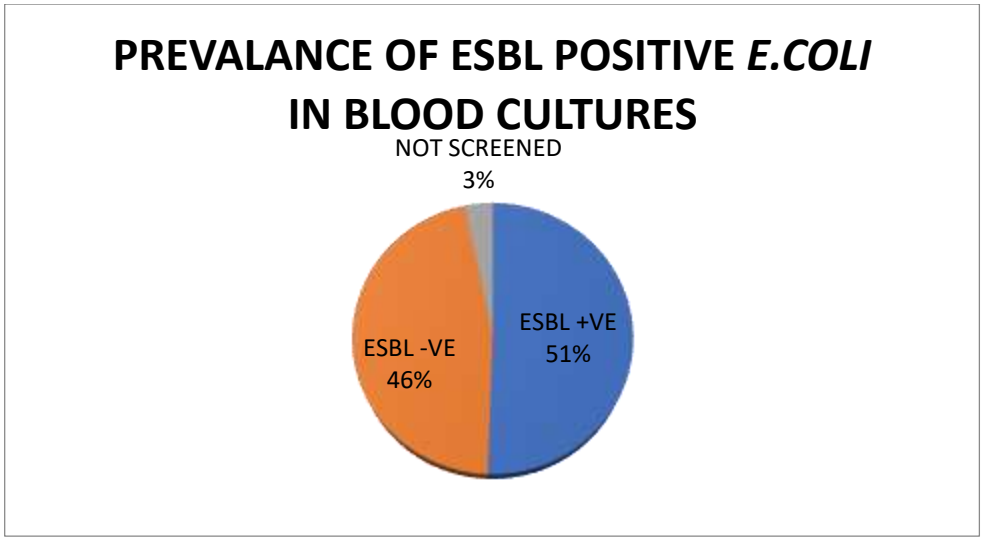

Figure 1 shows the prevalence of ESBL-positive *E.coli* isolated from bloodstream infections. The majority (46) 51% of blood cultures were positive for ESBL-positive. *E.coli* and 42(46%) were ESBL negative *E.coli.*

Effect of laboratory errors on the accurate and timely reporting of wound infections caused by extended-spectrum beta-lactamase positive Escherichia coli.

Prevalence of ESBL positive E.coli in wound infections.

Out of 85 samples positive for E.coli received by the lab between the periods of January 2019- march 2021, 44.71% (38) were ESBL positive while 55.29% (47) were ESBL negative.

Figure 2: Prevalence of ESBL-positive E.coli in wound infections. (N=85)

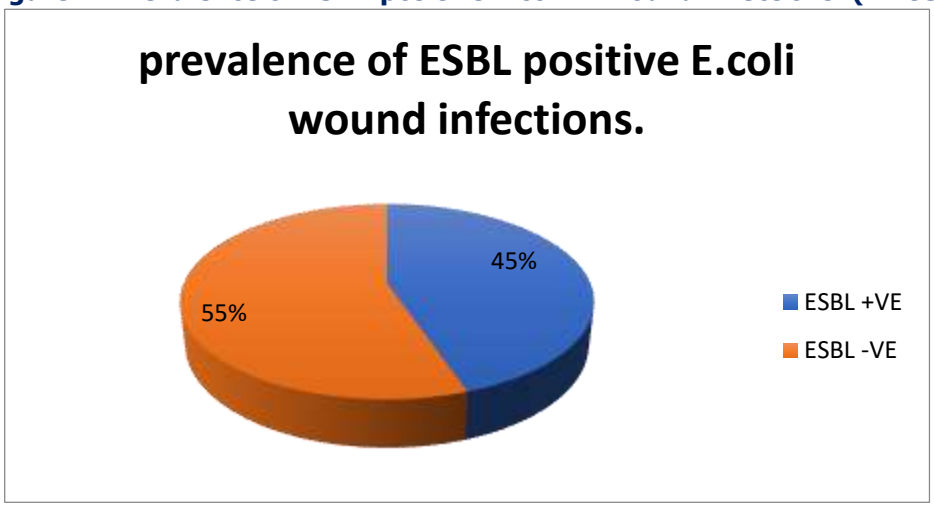

Figure 2 shows the prevalence of ESBL-positive *E.coli* isolated from wound infections. The majority 47 (55.29%) of pus swabs from wound infections were ESBL negative *E.coli* while 38(44.71%) were ESBL positive *E.coli.*

Discussion

Prevalence of ESBL-positive E.coli in bloodstream and wound infections

In our study, the prevalence of ESBL-positive *E.coli*was 50.55%, which was more than half of the 91 blood culture samples analyzed. The study findings are similar to a study done in China by Quan et al., 2017 at Zhejiang University where the prevalence of ESBL producing *E.coli*was 55.5% in bloodstream infections (Quan et al., 2017). However, in contrast to our study, the prevalence of ESBL-producing *E.coli*causing bacteremia in a study conducted by Kang et al in 2008 was lower (4.1%) (Kang et al in 2008). Additionally, the prevalence of ESBL-producing *E.coli* in another study conducted in South

Africa was much lower (29.9%) in bloodstream infections (Malande et al., 2019).

In our study, the prevalence of ESBL-positive *E. colicin* wound infections was 44.71% out of the total 85 samples isolated from pus swabs which was in contrast to the findings of a study conducted in Sudan where the prevalence of ESBL-positive *Escherichia coli in wound infections* was 55%, a higher prevalence in comparison to that discovered by our study (Ibrahim et al., 2013). Just like another study conducted in Ghana on Hospital Acquired Surgical Site Infections 38% of the bacteria isolated were *E.coli*and 50% of these were ESBL producing (Egyir et al., 2020).

Conclusion

50.55% of the isolated *E.coli* from bloodstream infections were ESBL positive as compared to wound infections, the prevalence was 44.71%. This could pose a great risk to the management of *E.coli*infections.

Recommendation

The laboratory should play the role of guiding physicians and medical personnel on the need for infection control measures to prevent the spread of ESBL *E.coli* organisms.

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List of Abbreviations.

E.coli; Escherichia *coli*
ESBL; Extended Spectrum Beta Lactamases
Expect; Extraintestinal pathogenic Escherichia coli
Lab; Laboratory
VF; virulence factor
MAC; MacConkey agar
BA; Blood agar
AST; Antimicrobial susceptibility test
Abx; Antibiotics

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Conflict of interest.

The author declares no conflict of interest.

Author Biography

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