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PREVALENCE OF THE DIFFERENT MICROBIOLOGICAL LABORATORY ERRORS THAT OCCUR IN PROCESSING BLOOD AND PUS SPECIMENS WITH ESBL POSITIVE E. COLI AT MAKERERE UNIVERSITY CLINICAL MICROBIOLOGY LABORATORY. A CROSS-SECTIONAL STUDY.

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Abstract

Background

Errors that occur in the diagnostic cycle of the microbiology laboratory cause a delay in the timely and accurate reporting of extended-spectrum beta-lactamase-positive*E. Coli* isolated from bloodstream and wound infections. Therefore, this study aimed to determine the Prevalence of The Different Microbiological Laboratory Errors That Occur in Processing Blood and Pus Specimens with ESBL-positive *E. Coli.*

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, A purposive sampling technique was used to select only records for which ESBL-positive *E.coli.*

Results

For Bloodstream infections: Out of these 91 request forms; the majority 61(67.03%) had no errors while 30 (32.97%) had errors. These included a missing date, time of reception, and initials of the recipient (60%), sex, age, and patient location (3.33%) missing patient contacts (10%), patient history, and source from which the sample was collected (6.67%). In 16.67% of the request forms physician's details were not indicated and in 13.33% antibiotics previously used were not indicated. **For wound infections;** Out of these 85 request forms; the majority 61 (71.76%) had no errors while the minority 24(28.24%) had errors. Some of the errors included missing date, time of reception of samples (33.33%), initials of recipient (33.33%), long collection date (4.167%), patient age (8.33%), patient location, no patient contacts (4.167%), no physician's details (8.333%) and no antibiotics previously used were not indicated (37.50%).

Conclusion

More errors were noted in the lab request forms for bloodstream infections (32.97%) as compared to the lab request forms for wound infections (28.24%).

Recommendation

The lab should work hand in hand with the ward to ensure lab request forms are properly filled out to curb the increasing errors in the lab diagnostic cycle.

Keywords; *E. Coli, Makerere University, wound infections, bloodstream infections, microbiological analytical errors, beta-lactamase enzymes.*
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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.). Therefore, this study aimed to determine the Prevalence of The Different Microbiological Laboratory Errors That Occur in Processing Blood and Pus Specimens with ESBL Positive *E. Coli* at Makerere University Clinical Microbiology Laboratory.

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

The participants of the study were selected based on inclusion and exclusion 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 errors that occur in the pre-analytical phase of processing blood specimens with ESBL-positive *E. coli.*

A total of 91 request forms were checked for any missing details that could affect the accurate and timely detection of bloodstream infections caused by ESBL-positive E.coli. Out of these 91 request forms; the majority 61(67.03%) had no errors while 30 (32.97%) had errors. These included a missing date, time of reception, and initials of the recipient (60%), missing collection date, sex, age, and patient location (3.33%) missing patient contacts (10%), mossing patient history and source from which sample was collected (6.67%). In 16.67% of the request forms physician's details were not indicated and in 13.33% antibiotics previously used were not indicated.

SJ Insight
Vol. 2 No. 1 (2025): January 2025 Issue
https://doi.org/10.51168/insights.v2i1.23
Original Article

Figure 1: Prevalence of pre-analytical errors that occur during processing blood specimens with ESBL-positive E.coli. (N=91)

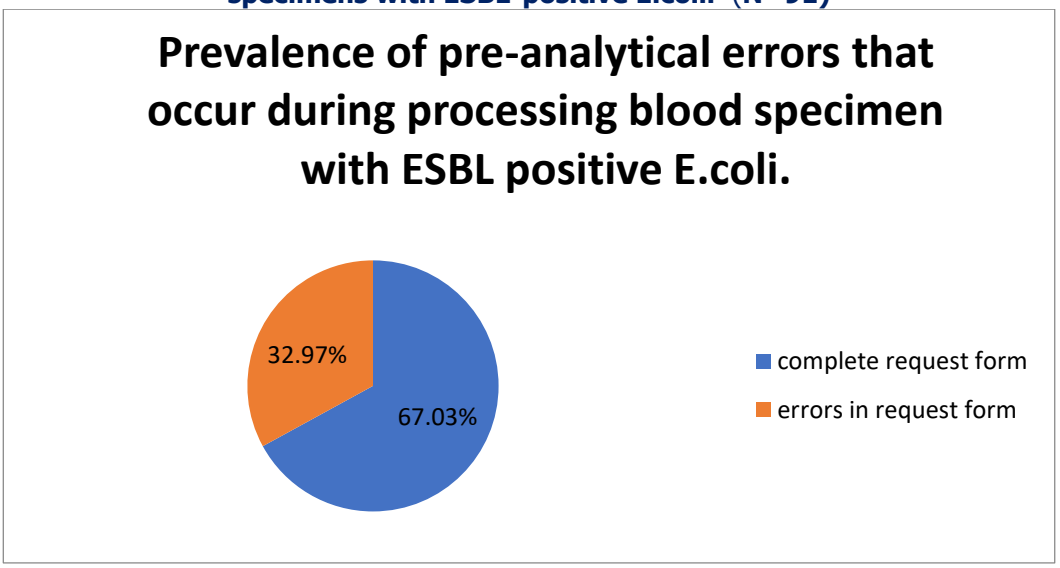


Figure 1 shows that the majority (67.03%) of the lab request forms were complete with no errors while a few 32.97% had missing details.

Table 1: Prevalence of the different pre-analytical errors that occur during processing blood specimens with ESBL-positive E.coli. (N=30)

Errors detected in the pre-analytical phase (lab request forms)	Number	Percentage
Missing date, time of reception of samples	18	60.00%
Missing initials of the recipient	18	60.00%
Missing collection date	1	3.33%
Missing sex of the patient	1	3.33%
Missing patient age	1	3.33%
Missing ward /patient location	1	3.33%
Missing patient contact	3	10.00%
Missing patient history	2	6.67%
Missing physician details	5	16.67%
Antibiotics previously used were not indicated	4	13.33%
No sample source indicated	2	6.67%

Table 1 above shows the prevalence of pre-analytical errors that occur while processing blood specimens with ESBL-positive *E.coli*. The majority of the samples 18(19.78%) had missing dates, time of reception, and recipient initials.

Prevalence of analytical errors in processing Blood specimens with ESBL-positive E. coli.

The lab bench books were checked for missing identification tests that could affect accurate and timely reporting of bloodstream caused by ESBL-positive E.coli. For All the 91samples (100%) gram stain, biochemical tests, and antimicrobial susceptibility tests were done. 5(5.49%) and 2(2.2%) of the samples received by the lab were not cultured on BA and MAC respectively.

SJ Insight
Vol. 2 No. 1 (2025): January 2025 Issue
https://doi.org/10.51168/insights.v2i1.23
Original Article

Figure 2: Prevalence of errors that occur in the analytical phase of processing blood specimens with ESBL positive E.coli. (N=91)

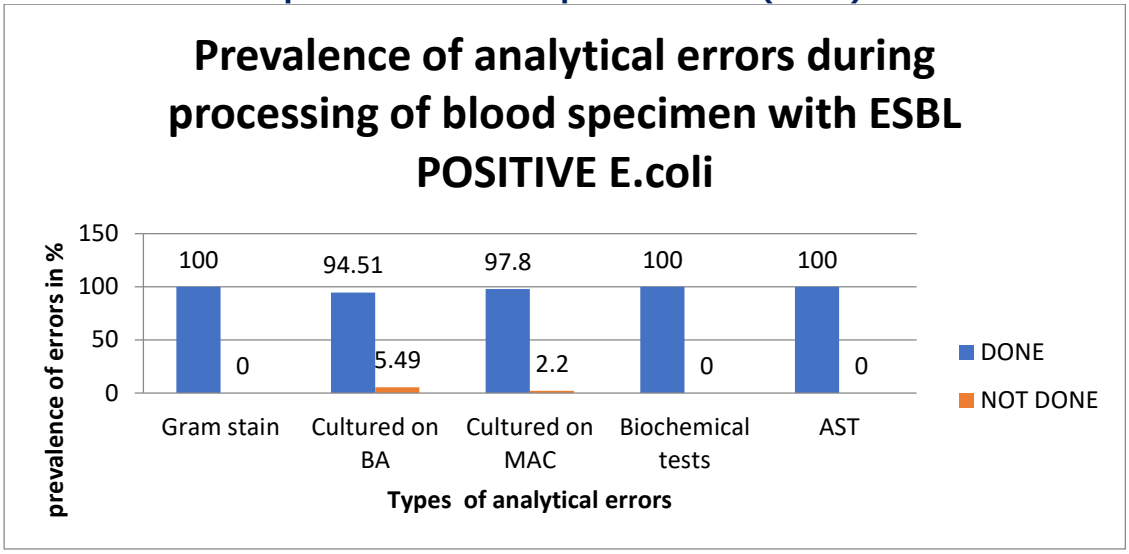


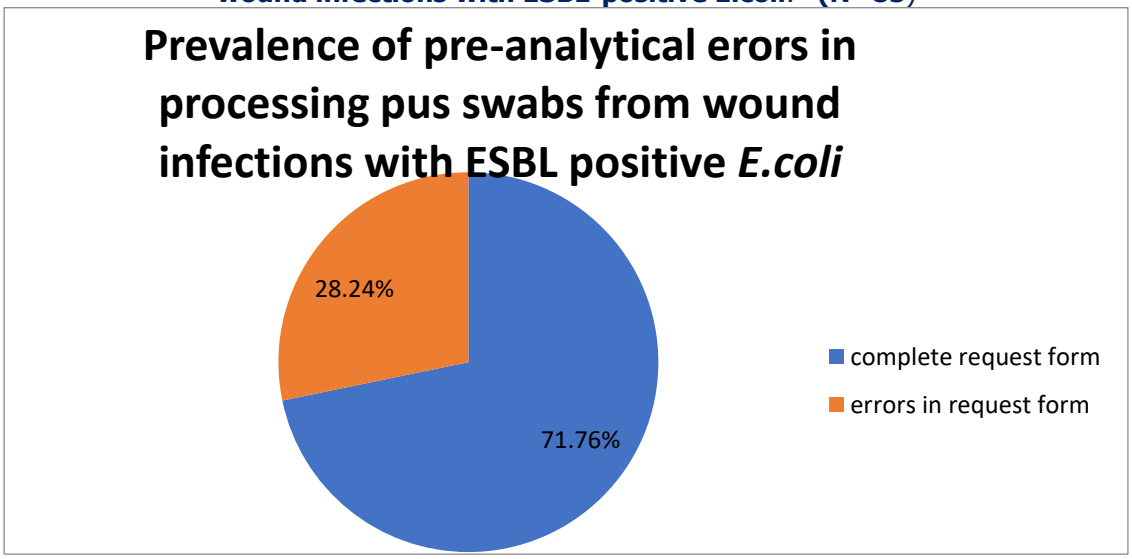
Figure 2 represents the different prevalence and types of analytical errors that occur during the processing of blood specimens with ESBL positive For All the 91 samples of wound infections caused by ESBL-positive E.coli in wound infections. Out of these 85 request forms; the majority 61 (71.76%) had no errors while the minority 24(28.24%) had errors. Some of the errors included missing date and time of reception of samples (33.33%), missing initials of recipient (33.33%), long collection date (4.167%), missing patient age(8.333%), missing patient location, no patient contacts (4.167%), no physician's details (8.333%) and no antibiotics previously used were not indicated (37.50%). (100%) gram stain, biochemical tests, and antimicrobial susceptibility tests were done. While 5(5.49%) and 2(2.2%) of the samples received by the lab were not cultured on BA and MAC respectively.

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

Prevalence of errors that occur in the pre-analytical phase of processing pus swabs from wound infections with ESBL-positive E.coli.

A total of 85 request forms were checked for missing details that could affect the accurate and timely detection

Figure 3: Prevalence of pre-analytical errors that occur during processing pus swabs from wound infections with ESBL-positive E.coli. (N=85)



SJ Insight
Vol. 2 No. 1 (2025): January 2025 Issue
https://doi.org/10.51168/insights.v2i1.23
Original Article

Figure 3 shows that the majority 61 (71.76%) of the lab request forms were complete with no errors and 30(28.24%) had missing details.

Table 2: Prevalence of the different types of pre-analytical errors that occur during processing pus swabs from wound infections with ESBL-positive E.coli. (N=24)

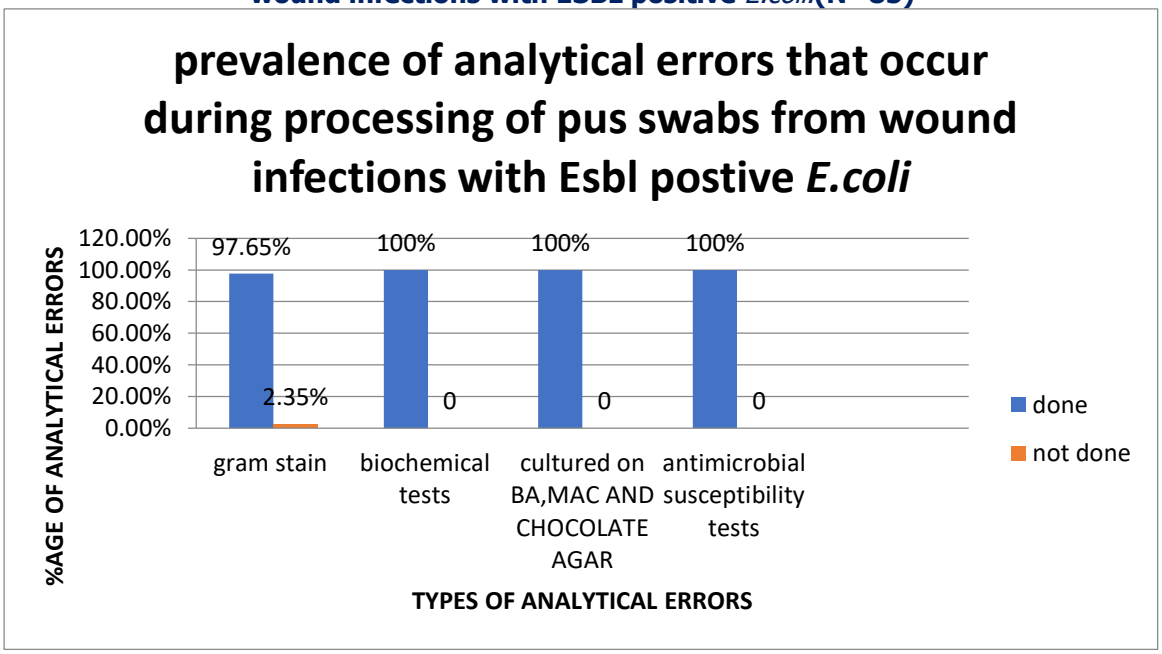
Errors detected in the pre-analytical phase of processing pus swabs (lab request forms)	Number	Percentage
Missing date, time of reception of samples	8	33.33%
Missing initials of the recipient	8	33.33%
long collection date	1	4.167%
Missing patient age	2	8.333%
Missing ward /patient location	1	4.167%
Missing patient contact	1	4.167%
Missing request form	1	4.167%
Missing physician details	2	8.333%
Antibiotics previously used were not indicated	9	37.50%

Table 2 shows the prevalence of pre-analytical errors that occur during processing pus swabs from wound infections with ESBL-positive *E.coli.* The majority of the request forms 9 (37.50%) did not indicate the antibiotic previously used and the minority.

Prevalence of analytical errors that occur in processing pus swabs from wound infections with ESBL positive E.coli.

The lab bench books were checked for missing identification tests that could affect the accurate and timely reporting of wound infections caused by ESBL-positive E.coli. For All 85 samples (100%) cultures on BA, MAC, and chocolate agar, biochemical tests and antimicrobial susceptibility tests were done. Only 2(2.35%) were not gram stained.

Figure 4: Prevalence of analytical errors that occur during processing of pus swabs from wound infections with ESBL positive *E.coli.*(N=85)



Discussion

Effect of pre-analytical errors on accurate and timely detection of ESBL positive E.coli in blood and wound infections.

Accurate and timely detection of ESBL-positive *E.coli* in blood and wound infections is highly dependent on a reliable laboratory diagnostic cycle, which, as part of the overall healthcare system, is prone to errors. Although numerous studies have been conducted on enhancing laboratory quality, the literature on errors in the lab diagnostic cycle is scarce. Evidence from recent studies done in Italy and Pakistan to determine the prevalence of lab errors that occur in the lab cycle demonstrates that a large percentage of laboratory errors occur in the pre-and post-analytical phase ((Communication, 2012; Plebani, 2010; State, 2015) These observations are confirmed by the findings in the present study where errors in the preanalytical (32.97%) and post-analytical (30.7%) process occurred much more frequently than in the analytical phase of processing blood specimen (7.69%). While processing pus swabs from wound infections prevalence of errors in the pre-analytical was 28.4%, analytical 2.35%, and post-analytical phase 38.65%. Errors in the pre-analytic phase arose from incorrect or incomplete information on the test request forms. In our study, 61(67.03%) of the blood request forms did not have any errors while 30(32.97%) had an assortment of errors which included missing collection date, sex, age, and patient location carrying 3.33%, missing patient contacts(10%), missing patient history and source from where the sample was collected(6.67%). In 16.67% of the request forms, there were physician details indicated and in 13.33%, antibiotics previously used were not indicated in the bloodstream infections. While in the wound infections, out of the 85 request forms revisited, 61(71.76%) did not have any errors while 24(28.24%) had errors. These errors include missing date and time of reception of samples (33.33%), missing initials of recipients(33.33%), long collection date(4.167%), missing patient age(8.333%), missing patient location, no patient contacts(4.167%), no physician's details (8.333%) and no antibiotics previously used indicated (37.50%).

These results contrast with the findings of a study done in Nigeria where 3.0% of forms did not state the gender of the patient, while 11.5% did not even give the age of the patient. 9.6% did not specify the patient's location and 34.0% did not have the patient's hospital number. 25.5% of forms also did not have the name of the attending Consultant and 15.5% did not have the name of the requesting Doctor, while 27.1% of all forms were not signed by the requesting Doctor. A diagnosis was not stated on 16.5% of forms. Also, the date of collection and nature of the specimen were not stated on 21.5% and 11.0% of forms respectively. Lack of important details such as the patient's name, sex, and age could affect accurate and timely detection of ESBL-positive *E.coli*. This, in turn, could increase morbidity as the wrong medication might be given to a patient and more time consumed in tracking the patient after an ESBL positive result has been obtained by the lab further delaying treatment.

In this study, 3.33% and 8.33%lab request forms for blood and wound infections respectively had no patient age. Similar to a study done by Oladiende and his colleagues where the age of the patients was either absent or inappropriately filled in 43% of the forms they analyzed.(Oladeinde et al., 2012) Missing details like age in the case of our study could potentially affect the accurate and timely detection of ESBL-positive *E.coli*, as certain laboratory indices are also age-dependent. Furthermore, in the African setting where patients may share names, the only quick identification is age, lacking parameters like age or even sex may lead to switching of results after diagnosis. Such incidents may lead to patients being administered wrong medication a direct link to increased drug resistance usually seen in overconsumption of drugs in an attempt to cure the misdiagnosed infection.

In this study 16.7% and 8.33% of the request forms for blood and wound infections respectively physician details were not indicated. This was similar to the findings of State et.al where 2.5% of forms did not have the names of the attending Consultant, 15.5% did not have the names of the requesting Doctor and 27.1% of forms did not carry the signatures of the requesting Doctor. (State, 2015) Often, results are sent to the wrong Doctor because such forms have not been properly filled by the requesting Doctors.

In this study, 6.67% of the request forms for blood cultures had no patient history similar to the findings of Oladeinde et. al in which a total of 151 (6.4%) of forms did not carry a diagnosis or patient history. The absence of a working diagnosis often leads to extraneous and unnecessary additional tests that delay turnaround time.(Oladeinde et al., 2012)

Antibiotics previously used by the patients were not indicated in 13.33% and 37.50% of blood culture and wound swab request forms respectively, which could potentially result in wrong results regarding the antimicrobial susceptibility pattern of the isolated organism (Seol et al., 2013).

The date, time, and initials of the recipient of the sample were not indicated in 60% and 33.33% of blood culture and wound swab request forms. A higher percentage compared to the findings of Plebani et.al in which 2.5% of samples were not received In the lab information system. (Carraro & Plebani, 2007)The absence of these derives from poor compliance with sops that ensure quality control such that samples received meet the lab acceptance criteria and no one can be held accountable for errors that occur during this stage of sample reception.

In this study 6.67% of lab request forms for blood cultures had no specimen source indicated. This error rate is much higher compared to the 2.7% obtained by a study done by Oladeinde et al. Absence of information regarding the type of sample collected, bloody pleural aspirate or cerebrospinal fluid can easily be taken for blood by the laboratory staff, resulting in the use of inappropriate diagnostic techniques, reference ranges, and ultimately misleading results (Muluberhan Ali, 2019)

Effect of analytical errors on accurate and timely detection of ESBL positive *E.coli* in blood and wound infections.

Avery's low error rate was found in the analytical phase and the majority of the samples were processed following the lab's SOPS.100% of blood culture samples had gram stain, biochemical tests, and antimicrobial susceptibility tests were done. While 5(5.49%) and 2(2.2%) of the samples received by the lab were not cultured on BA and MAC respectively. In the case of pus swabs obtained from wound infections, all 85 samples (100%) were cultured on BA, MAC, and chocolate agar, biochemical tests and antimicrobial susceptibility tests were also done. Only 2(2.35%) were not gram stained. The results of this study are confirmed by other studies where the lowest error rates were found in the analytical phase.(Carraro& Plebani, 2007) These errors were attributed to failure to follow standard operating procedures and assay instructions(Schultze& Irizarry, 2017)

Conclusion

Lab errors were greatest in the pre-analytical (32.97%) and post-analytical (30.7%) phases as compared to those in the analytical phase during processing blood cultures (7.69%). This was also similar in request forms for wound infections, in which the prevalence of lab errors in the pre-analytical (28.4%) and post-analytical phase (38.65%) was greater than those in the analytical phase (2.35%). All the lab request forms contained adequate information as required however some had missing dates, time of reception, and initials of the recipient (60%), missing collection date, sex, age, and patient location (3.33%) missing patient contacts (10%), mossing patient history and source from which sample was collected (6.67%). In 16.67% of the request forms physician's details were not indicated and in 13.33% antibiotics previously used were not indicated. Such factors could delay the delivery of results to the patient.

Recommendation

The lab should work hand in hand with the ward to ensure lab request forms are properly filled out; sops are followed meticulously during processing blood and pus swab samples and results are released on time to curb the increasing errors in the lab diagnostic cycle.

Acknowledgment

We wish to express my deepest gratitude to the people who have extended their assistance for the success of this study, the Almighty God, the giver of life, knowledge, and wisdom, Dr.Margaret Lubwama for her valuable knowledge, expertise, and guidance that made the completion of this work successful and lastly to our classmates that shared their knowledge and ideas in the construction of the report. God bless you all.

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

Source of funding.

The study was not funded.

Conflict of interest

The author declares no conflict of interest.

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