Original Research Article

An etiology of Sputum Culture Contamination and their Drugs Susceptibility Patterns among TB-suspected Patients at Epicentre Mbarara Research Centre, Uganda

ABSTRACT

Aims: Contamination in TB cultures is problematic as it allows the growth of non-target bacterial or fungal species in sputum samples, potentially concealing *M. tuberculosis*. This study aimed to determine the prevalence, characterization, and drug susceptibility patterns of contaminants in sputum cultures from TB-suspected patients.

Methods: A laboratory-based cross-sectional study was conducted from October 2023 to June 2024 at the Epicentre Mbarara Research Centre, involving 81 sputum samples from TB-suspected patients. Conventional decontamination procedures were used, and both solid Lowenstein Jensen (LJ) and liquid Mycobacterium Growth Indicator Tube (MGIT) culture methods were applied. Standard culture techniques and physicochemical analyses were employed. Bacteria were incubated at 37°C in O_2 and Co_2 incubators, with growth observed within 24 to 48 hours and up to three days for yeast contaminants. Sabouraud Dextrose Agar cultures were monitored for two weeks for filamentous fungi. Antibiograms for bacterial isolates were determined using the Kirby-Bauer, while broth microdilution was used for fungal isolates.

Results: Of 81 samples, 14(17.28%) were contaminated. The most common bacterial contaminants were *Staphylococcus aureus, Bacillus subtilis*, *Streptococcus viridans, Staphylococcus epidermidis*, and *Escherichia coli*. Fungal contaminants included *Aspergillus flavus*, *Penicillium species*, *Candida albicans, Cryptococcus neoformans*, and *non-albican Candida species*. High resistance to erythromycin (73%) and tetracycline (50%) was noted among bacterial isolates, with sensitivity to vancomycin (67%), gentamycin (60%), and chloramphenicol (58%). Yeast isolates showed (87.5%) susceptibility to itraconazole and (75%) to amphotericin B but (50%) resistance to 5-flucytosine. Mold isolates exhibited (100%) susceptibility to itraconazole, amphotericin B, and 5-flucytosine but complete resistance to caspofungin.

Conclusion: The level of contamination in TB cultures was noted, and *Staphylococcus aureus and Candida albicans* were observed as the most common contaminants. Bacterial isolates resisted erythromycin and tetracycline, while fungal isolates were generally susceptible to itraconazole and amphotericin B. We recommend Strengthening decontamination and monitoring common resistant contaminants.

Keywords: Bacterial, Fungal contaminants, TB-suspects

1. INTRODUCTION

Tuberculosis (TB) is a global health challenge with 10.6 million new cases in 2021, leading to 1.6 million deaths [1]. Drug-resistant TB, particularly multi-drug resistant (MDR) and extensively drug-resistant (XDR) TB, complicates treatment and control efforts. Uganda is

one of the 30 high-burden countries for TB and TB/HIV co-infection, with an incidence rate of 198 per 100,000 population[2]. MDR-TB prevalence in Uganda is estimated at 4.4%, with XDR-TB prevalence being a critical concern [3]. In response to the WHO updated guidelines for managing DR-TB, Uganda introduced community-based directly observed therapy (CB DOT) in October 2020. This shift allowed for the use of shorter, more patient-friendly effective, all-oral treatment regimens than previous injectable options. CB DOT has been associated with improved adherence rates and reduced patient travel-related burdens, thereby enhancing overall treatment success [4]. In Uganda, tuberculosis (TB) diagnosis uses both phenotypic and genotypic methods, with a focus on Loop-Mediated Isothermal Amplification (LAMP) technology. Unlike traditional smear microscopy, TB-LAMP offers a rapid and reliable alternative with higher sensitivity and specificity [5]. *Mycobacteria* cultivation, on the other hand, is the only technique for distinguishing between bacterial cells that are alive and deceased [6].In addition, culture methods have demonstrated high TB diagnostic sensitivity and specificity, making them the gold-standard diagnostic approach [7]. Furthermore, using liquid culture (Mycobacterium Growth Indicator Tube) increases the number of bacteriologically confirmed tuberculosis cases by 20% - 30% [8]. Despite this, the tuberculosis culture is not widely available due to the high cost and laboratory biosafety infrastructure requirements. Furthermore, the TB culture method remains suboptimal due to contamination issues, which impede mycobacterium isolation despite existing laboratory decontamination procedures [9]. Decontamination procedures may increase the possibility of contamination due to time or concentration constraints, poor specimen quality, delays in transport to the lab, and Inadequate standards of medium and incubation related to a culture condition may be cited as potential sources of contamination and culture contamination caused by *S. pyogens, S. aureus, E. coli, K. pneumoniae, P. aeruginosa A. fumigatus,* and *C. albican* which decreases the proportion of interpretable results and the diagnostic utility of culture systems [10]. There are increasing cases of TB patients having a co-existence of *Mycobacteria tuberculosis* and other organisms in the pulmonary niche these could be easily dismissed as contaminants yet they could be critical opportunistic pathogens exacerbating the TB, and the associated organisms may be key opportunistic infections that require critical isolation for better patient care. Tuberculosis sputum culture contamination is caused by various microorganisms, that survive the lab pre-culture decontaminated method and some may be resistant to currently available antibiotics and antifungal agents [11]. Unfortunately, little is known about the specific contaminants and drug susceptibility patterns of the isolated contaminants at the Epicentre Mbarara Research Centre TB laboratory and the risk of contamination complicates TB diagnosis and hurts treatment decisions. Therefore, the purpose of this study is to investigate the etiology of sputum culture contamination and drug susceptibility patterns among TB-suspected patients at the Epicentre Mbarara Research Centre, Uganda.

2. MATERIAL AND METHODS

2.1 Study design

This was a laboratory-based cross-sectional study, in which the contaminated TB cultures were investigated for etiology and their drug susceptibility pattern of contaminants microorganisms.

2.2 Study site

The research was carried out at the Epicentre Mbarara Research Centre, Phenotypic characterization of isolated bacteria was done at the Epicentre Laboratory, and Fungal isolation was done at the Mycology Unit of Microbiology at Mbarara University of Science and Technology in Mbarara, Uganda. Epicentre Mbarara Research Centre, founded by Médecins Sans Frontières (MSF), is housed within Mbarara University of Science and Technology, it has a biosafety level 3 that can perform Tuberculosis diagnostic activities such as microscopy, Lowenstein-Jensen, MGIT media, and antibacterial sensitivity patterns using Gene Xpert and Line probe assays. The laboratory is at the top of the hierarchical TB culture laboratory network, and it is supported technically by the supranational National Reference TB Laboratory and the Antwerp Institute of Tropical Medicine. Qualogy accredited the Laboratory for Good Clinical and Laboratory Practices. This laboratory is critical to conducting numerous TB studies because it is the only TB culture laboratory in south and western Uganda making it ideal for this study because samples were collected on-site and processed in real-time, reducing the risk of contamination from the sample shipment process.

2.3 Study Population

The study was conducted on all the sputum samples of suspected TB patients undergoing culture for routine diagnostic processes and TB treatment follow-up culture diagnosis at the Epicentre Mbarara Research Centre.

2.4 Section criteria

2.4.1 Inclusion criteria

- All sputum samples collected from suspected TB patients at Epicentre Mbarara Research Centre were included.

2.4.2 Exclusion criteria

- Sputum samples with insufficient volume, damaged, salivary sample, leakage, and missing laboratory request form

2.5 Sample Size Estimation

The sample size was calculated using the Kish and Leslie formulation [12]

$n = Z^2 P (1 – P) / d^2$

Where,

n = sample size

Z = critical value that corresponds to 95% confidence interval

P = proportion of contamination. The prevalence of 10.4% of sputum culture contamination according to [10] was considered

d = margin of error which corresponds to 0.05.

$n = \frac{1.96 \times 1.96 \times 0.104(1-0.104)}{0.0025} = 143$ participants

Using the above information in the above formula, a sample size of 143 TB-suspected patients was calculated.

When we corrected the sample size for a finite population, since at EMRC, about 75 sputum samples are cultured per year, so the calculated sample size above was corrected for a finite population with the formula [13]

$n = \frac{n_0}{1+n_0/N} = \frac{143}{(1+143)/75} = 74$ participants

Considering the 10%, factors of the isolates that may not grow, our sample size was a minimum of 81 participants.

2.6 Sample collection

Sputum specimens were acquired from TB-suspected patients at the Epicentre Mbarara Research Centre using the standard sputum collection protocol, hence a total of 81 sputum samples were enrolled in this study.

2.7 Laboratory Methods

2.7.1 Conventional Decontamination Procedure

We used 2.5% NaOH to achieve decontamination and the procedures were performed following the standards operating procedures. The sputum specimens were mixed with an equal amount of sodium hydroxide, vortexed, incubated, and centrifuged for 20 minutes. The supernatant was reconstituted in PBS and inoculated into PANTA-supplemented Mycobacterium Growth Indicator Tube and Lowenstein Jensen media [10]. And incubated in an automated BACTEC MGIT 960 system. Nine contaminated MGIT cultures where non-mycobacterial microorganisms grow, with no acid-fast bacilli (AFB) on ZN smear but growth on BA or SDA were further processed. Lowenstein Jensen slants were placed in an incubator at 37^{o}C for up to 8 weeks. Five contaminated LJ slope cultures where non-mycobacterial microorganisms grow have a visible colony of non-acid-fast bacteria, a change in the color of the LJ media, and a crack or liquefied in the LJ media were further followed up for identification as indicated in Figure 1.

2.8 Culture and isolation of bacterial contaminants

In our study, Contaminants were isolated by subculturing onto duplicated blood agar supplemented with 5 % (v/v) defibrinated sheep blood, incubated at 37°C with 5% CO_2, and

examined for bacterial growth, hemolytic patterns, and colony morphology were captured as previously described by (McClean *et al*,.2011). Gram staining characteristics and conventional biochemical tests were used to identify and classify contaminants encountered in those cultures. Gram-positive cocci and Gram-positive rods were identified using Catalase, DNase, Coagulase, Urease, SIM, Citrate test, Novobiocin, Optochin, and Penicillin sensitivity. Gram-negative rods were identified using the Sulfur Indole motility (SIM) test, Triple sugar iron agar (TSI), Urease, and Citrate utilization tests. After the confirmation, all the plates and tubes were disposed of properly by autoclaving and then incineration, as per laboratory waste disposal protocols. These preliminary identification methods were conducted at the Microbiology unit of Epicentre Mbarara Research Centre Laboratory.

2.9 Culture and isolation of fungal contaminants

The cottony colonies from the contaminated LJ cultures or positive tubes of MGIT were presumed to be contaminated with filamentous fungi and were inoculated on duplicated Sabouraud dextrose agar. The inoculated plates were incubated at different temperatures to encourage fungal growth, and they were checked every day to see if mold was growing at 20-30^{0}C for two weeks and if the yeast was growing at 37^{0}C for three days as previously described by [14]. Viable yeast and mold cultural isolates were identified by observing colony morphology, plates showing no growth were considered negative and disposed of properly by autoclaving and then incinerating, per laboratory waste disposal protocols. Molds were identified by looking at their colonies microscopic and macroscopic features, and culture elevation, consistency, rate of development, and coloration on both the front and back were noted. Microscopic confirmation of mold isolates such as *Aspergillus flavus*, and *Penicillium species* was prepared using the tease-mount technique and stained with lactophenol cotton blue as described by [15]. At a 40x magnification, microscopic characteristics were observed, including the development of conidia of long thread-like structures called hyphae, which collectively form a mycelium, brush-like spore structures, shapes, and arrangement of phialides were noted to help in the identification of the mold isolates. The initial method used to identified yeast isolates was via their morphological features in primary SDA cultures which showed white color, smooth and shiny colonies, and subsequently confirmed by Gram stain morphology. Germ tube formation and CHROMagar media were done to differentiate the *Candida albicans* and *Non-candida species,* and the India Ink, urease test was used to identified *Cryptococcus neoformans* [16]. The confirmation was conducted courtesy of support from the Mycology Unit of Microbiology at Mbarara University of Science and Technology in Mbarara, Uganda

2.10 Antimicrobial susceptibility test of bacterial and fungal isolates

The standard disc diffusion technique was used to test the in-vitro susceptibility of bacterial isolates to different antibacterial drugs as per standard [17]. Three colonies from a 24-hour pure culture plate of each type of bacterial growth were chosen using a sterile wire loop and

then emulsified in five milliliters of sterile normal saline. The tube containing the bacterial suspension was placed into a sensitive nephelometer (TREK Diagnostic Systems, UK) and adjustments were made until the 0.5 McFarland standards were achieved. A sterile cotton swab was used by dipping it into the inoculum and the excess medium was removed by pressing the swab onto the wall of the tube. The entire surface area of the Mueller-Hinton agar plates was swabbed completely by rotating the plate and allowing the plates to dry for five minutes so that the medium absorbs the inoculum properly before applying antibacterial drugs to the plates [18]. The following antibiotics were tested: chloramphenicol (30µg), erythromycin (15µg), Tetracycline (30 µg), Gentamicin (10 µg), Trimethoprim (5µg), Vancomycin 30µg, Ciprofloxacin (10 µg) as guided by CLSI M100. Place sterile paper discs containing specific concentrations of various antibiotics onto the inoculated agar surface at a distance of 24mm using sterile forceps. The inoculated plates were incubated for 24 hours at 37ºC using an incubator. After incubation, the diameters of the zones of inhibition around each antibiotic disc were measured using a ruler, and the isolates were then categorized as sensitive, intermediate, or resistant as per the CLSI M100. Antifungal susceptibility testing was performed in compliance with the Clinical and Laboratory Standards Institute M27-A3 and M38-A2 [19, 20] for molds and yeasts with optical densities of the suspensions adjusted to the opacity of 0.5 McFarland standards, respectively. Antifungal stock solutions were prepared as follows: Caspufingin and 5-flucytosine were dissolved in sterile distilled water, and amphotericin B, and itraconazole were suspended in analytical-grade dimethyl sulfoxide. In this investigation, dilutions ranging from 0.03 to 16µg/ml were made. The susceptibility test of the isolates was achieved using RPMI-1640 supplemented with glutamine, phenol red, and 0.2% glucose. For the broth microdilution, this was achieved using sterile 96-well microtitre plates and 100µl suspension of the test organism, RPMI, and antifungal drugs were applied to each well, and 100µl of the drug solvent and 0.5 McFarland standard suspension of *Candida albicans* ATCC 10231 were added to the growth control wells as previously described by [15, 21]. The plates were incubated for a maximum of 72 hours at 37°C in an incubator. The Minimal Inhibitory Concentration was read as the lowest antifungal concentration that prevents the development of the tested isolates. Using Microsoft Excel, STATA version 17 was used to determine the proportion of sputum cultures that were contaminated out of the total cultures examined, and corresponding 95% confidence interval, descriptive statistics in the form of graphs, tables, and percentages were generated.

Flow Chart of Experiments in the Study

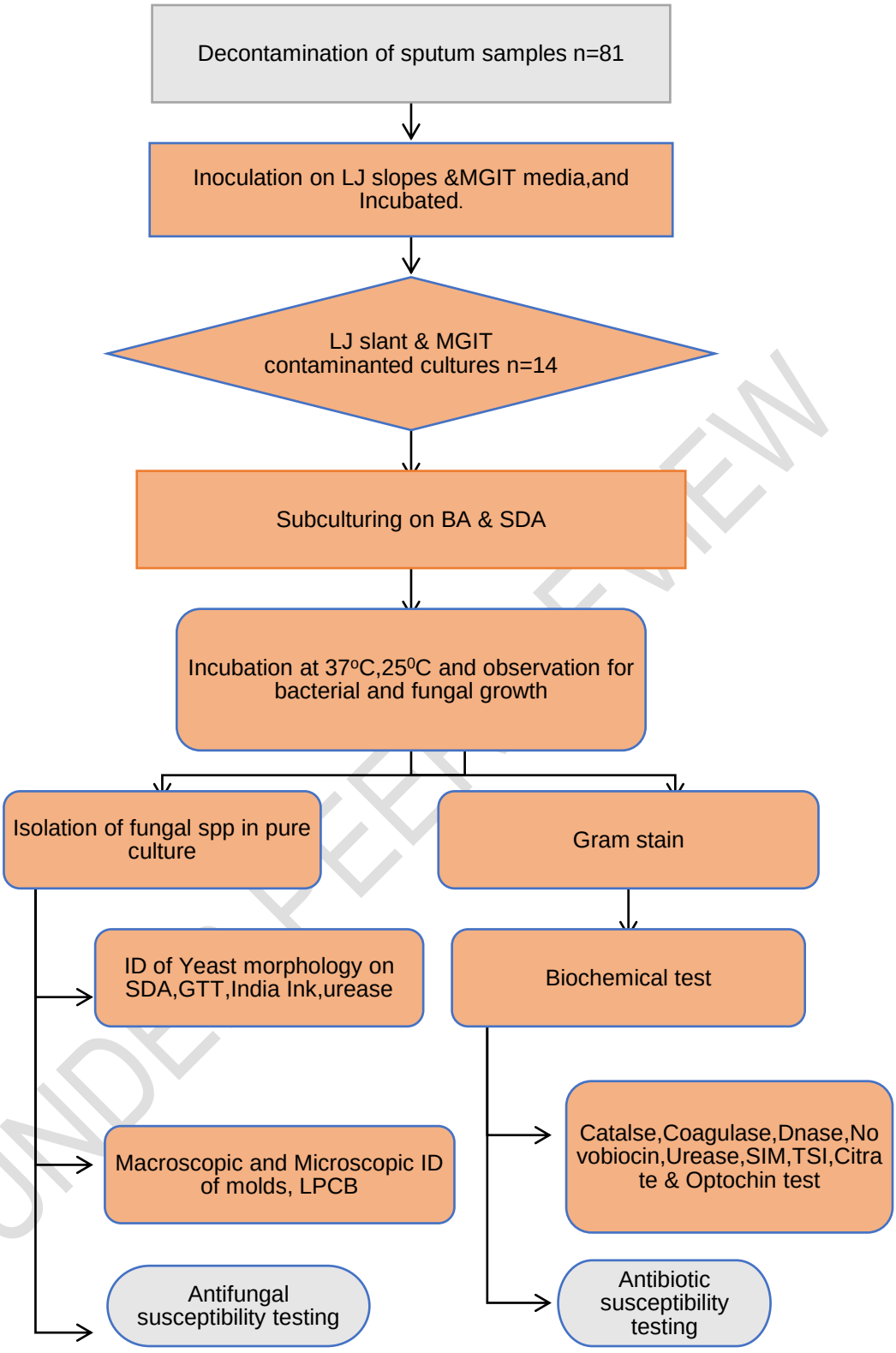

Figure 1:The process diagram illustrates how 81 samples from patients suspected of TB in Mbarara, Uganda, were used to isolate bacterial and fungal contaminants in tuberculosis cultures.

3. RESULTS

Among the 81 sputum samples cultured from tuberculosis-suspected patients at the Epicentre Mbarara Research Centre, the majority were males (60.5%) and the predominant age group was 26-35 years (33.3%). Most sputum samples appearance was a mucoid (61.7%), and a large portion was from patients undergoing TB screening (64.2%), generally, the overall prevalence of sputum culture contamination was 17.28 % (95%CI: 10.4 - 22.22). Among the bacterial contaminants, *Staphylococcus aureus* and *Bacillus subtilis* were the most common organisms. Fungi isolated included *Candida albicans* being the most prevalent and *Cryptococcus neoformans*, *non-albicans Candida* and *Penicillium* species, and *Aspergillus flavus* were the least common isolates as shown in Figure 2 below.

Table 1 shows the antibiotic susceptibility testing, *Staphylococcus aureus* 5(72%) showed susceptibility to Ciprofloxacin, Gentamycin, and Chloramphenicol, while 7(100%) were resistant to Tetracycline. *Staphylococcus epidermidis* 1(50%) of isolates showed susceptibility to Ciprofloxacin and Gentamycin, but all isolates exhibited 2(100%) resistance to Chloramphenicol, Trimethoprim, and Erythromycin, while 2(100%) displayed intermediate to Tetracycline. *Streptococcus viridans* indicate that 2(100%) of the isolates were susceptible to Vancomycin and Chloramphenicol, and all isolates 2(100%) of *S. viridans* were resistant to Trimethoprim. *Escherichia coli* showed high resistance to Ciprofloxacin (100%) but was 1 (100%) intermediate to the other four drugs.

Table 2 shows the susceptibility patterns of various antifungal agents against yeast and filamentous species. *Candida albicans* exhibited the highest sensitivity to Itraconazole (83%) and the highest resistance to Amphotericin B (33.3%). *Non-albicans Candida spp* showed 100% sensitivity to all tested drugs and *Cryptococcus neoformans* displayed 100% sensitivity to Itraconazole, Amphotericin B, and Caspofungin, but 100% resistance to 5-Flucytosine.For filamentous isolates, *Aspergillus flavus* and *Penicillium spp* both showed 100% sensitivity to Itraconazole, Amphotericin B, and 5-Flucytosine, but 100% resistance to Caspofungin.

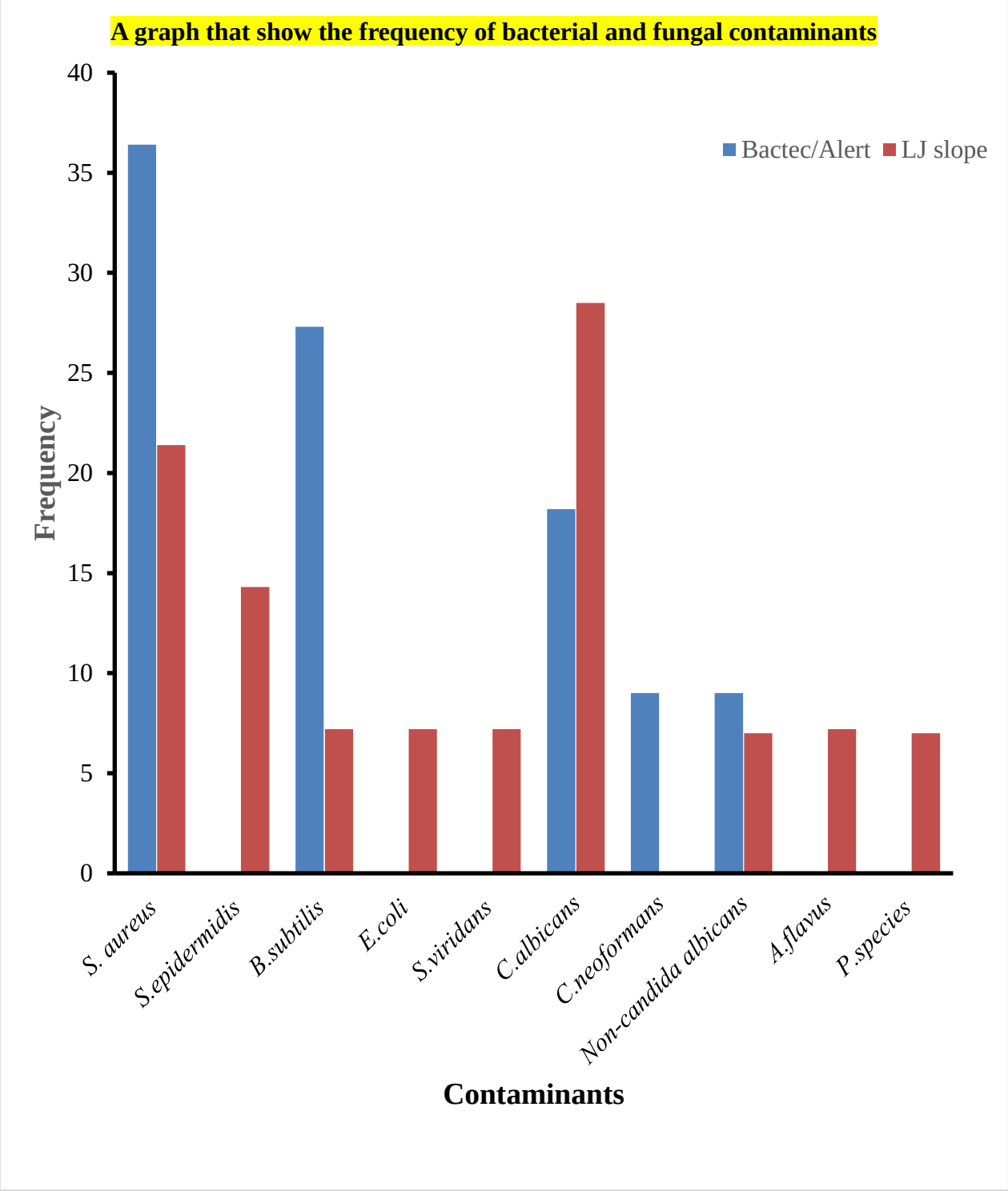

Figure 2: The frequency of bacteria and fungi identified from contaminated MGIT and LJ slant medium

Table 1: Antibiotic susceptibility pattern of isolated bacteria from TB sputum culture contaminations

Bacterial isolates	No	Patterns	Antibiotic agents tested						
			CIP	CN	VAN	TMP	E	TTC	C
			No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S. aureus	7	S	5 (72)	5 (72)		4 (57)	1(14)	0 (0)	5 (72)
		R	1 (14)	2 (28)	*	2 (29)	5(72)	7 (100)	1 (14)
		I	1 (14)	0 (0)		1 (14)	1(14)	0 (0)	1 (14)
S. epidermidis	2	S	1 (50)	1 (50)		0 (0)	0 (0)	0 (0)	0 (0)
		R	0 (0)	1 (50)	*	2 (100)	2(100)	0 (0)	2 (100)
		I	1 (50)	0 (0)		0 (0)	0 (0)	2 (100)	0 (0)
S. viridans	2	S	1 (50)		2(100)	0 (0)	1 (50)	1 (50)	2 (100)
		R	0 (0)	*	0 (0.0)	2 (100)	1 (50)	0 (0)	0 (0)
		I	1 (50)		0 (0.0)	0 (0)	0 (0)	1 (50)	0 (0)
E. coli	1	S	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)
		R	1 (100)	0 (0)	*	0 (0)	*	0 (0)	0 (0)
		I	0 (0)	1(100)		1 (100)		1 (100)	1 (100)
Overall	16	S	9 (56.25)	6 (60)	4 (67)	4 (33)	3 (20)	4 (25)	7 (58)
		R	4 (25)	3 (30)	2 (33)	6 (50)	11 (73)	8 (50)	3 (25)
		I	3 (18.75)	1 (10)	0 (0)	2 (17)	1 (7)	4 (25)	2 (17)

*Key: Numbers in brackets represent percentage (%) of total bacterial species per categorized patterns. CIP: Ciprofloxacin, CN: Gentamycin, TTC: Tetracycline, VAN: Vancomycin, TMP: Trimethoprim, E: Erythromycin, C: Chloramphenicol, S: Sensitive, R: Resistant, I: Intermediate, Untested antibiotic is suggested by **

Table 2: Antifungal susceptibility patterns of isolated yeasts and filamentous fungi from TB sputum culture contaminations

Yeast isolates	No	Patterns	Antifungal agents tested			
			ITZ	AMB	CAS	FCY
			No (%)	No (%)	No (%)	No (%)
C. albicans	6	S	5 (83)	4 (66.7)	1 (17)	3 (50)
		R	1 (17)	2 (33.3)	2 (33)	0 (0)
		I	0 (0)	0 (0)	3 (50)	3 (50)
Non albican candida spp	1	S	1 (100)	1 (100)	1 (100)	1 (100)
		R	0 (0)	0 (0)	0 (0)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)
C. neoformans	1	S	1 (100)	1 (100)	1 (100)	0 (0)
		R	0 (0)	0 (0)	0 (00)	1 (100)
		I	0 (0)	0 (0)	0 (0)	0 (0)
Overall	8	S	7 (87.5)	6 (75)	3 (37.5)	4 (50)
		R	1 (12.5)	2 (25)	2 (25)	4 (50)
		I	0 (0)	0 (0)	3 (37.5)	0 (0)
Aspergillus flavus	1	S	1 (100)	1 (100)	0 (0)	1 (100)
		R	0 (0)	0 (0)	1 (100)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)
Penicillium spp	1	S	1 (100)	1 (100)	0 (0)	1 (100)

		R	0 (0)	0 (0)	1 (100)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)
Overall	2	S	2 (100)	2 (100)	0 (0)	2 (100)
		R	0 (0)	0 (0)	2 (100)	0 (0)
		I	0 (0)	0 (0)	0 (0)	0 (0)

Key: Numbers in brackets represent percentage (%) of total fungal species per categorized patterns' Itraconazole, AMB, Amphotericin B, CAS, Caspofungin, FCY, 5-flucytosine

4. DISCUSSION

In our study conducted at Epicentre Mbarara Research Centre, the prevalence of TB sputum culture contamination was 17.28% (95%CI 10.4-22.22). This prevalence is higher than the finding by [22] in India reported a lower contamination rate of 5.6%, which might be attributed to the variations in the local environment, such as climate and microbial flora. However, our finding is comparable with contamination reported in Northern Ireland 10% [23], Tanzania 12% [24], Ethiopia 22 % [25], Uganda 10.4% [10]. But was lower than what was reported in studies conducted in Botswana 35.6% [26], and Burkina Faso 40% [11]. Though no mycobacterial culture technique is completely free of contaminants, aseptic methods and appropriate decontamination protocols such as chlorhexidine which was not applied in the current study can reduce the number of contaminants and enhance the detection of *Mycobacterium tuberculosis* in culture [27]. Additionally, the increase in breakthrough contaminants in our study raises concerns about patient management and infection control. The reasons remain unclear, but one possibility is that the background microbial flora has become more resistant or the concentration of antibiotic-selective agents supplemented in solid and liquid TB cultures to suppress the growth of contaminants was too low, hence this is the area for further study to determine the MIC of antibiotics required to inhibit the growths of contaminants. *Staphylococcus aureus, Staphylococcus epidermidis*, *Bacillus subtilis, and Escherichia coli* were the most common bacterial contaminants found in this study. *Candida albicans, Cryptococcus neoformans, Penicillium species, and Aspergillus flavus* were the fungal contaminants. *Staphylococcus aureus*, found in this study as the most common, is similar to a study conducted in Northern Ireland, UK, which found that *Staphylococcus epidermidis* was the most frequently isolated contaminant [23]. Both identified Gram-negative and Gram-positive bacteria from LJ slants and MGIT cultures in this study align with the results of [28] in Uganda, except for the absence of *Streptococcus pyogenes*. Additionally, [29, 30] reported *Staphylococcus* species in antibiotic-free LJ culture tubes from patients at Mbarara Regional Referral Hospital, and in a French hospital. This points to the fact that the organisms found in this study are some of the most prevalent contaminants found in cultures of *M. tuberculosis*. For efficient and high-quality results, the current MGIT and LJ TB culture methods must be used while being aware of potential emerging contaminants, this is because some organisms found were typical flora,

suggesting the failure of the chemical decontamination step is indicated by the presence of contaminating bacteria and fungi. The N-acetyl-L-cysteine (NALC)/NaOH method is the most commonly used approach for isolating *Mycobacterium* from tuberculosis (TB) patients. A more effective method involves a two-step decontamination process using NALC/NaOH and oxalic acid. This technique significantly reduces the contamination rate in sputum cultures and allows for better recovery of mycobacteria compared to using NALC/NaOH alone. However, the NALC/NaOH/oxalic acid method may affect the viability of mycobacteria, potentially leading to false-negative results, particularly in samples with low mycobacterial counts. While using NaOH alone is cost-effective and efficient, the precise timing of the decontamination process is crucial to avoid unnecessarily killing mycobacterial organisms. The NALC/NaOH decontamination method can be used with caution, ensuring optimized protocols to balance contamination control with the preservation of mycobacterial viability. *Aspergillus flavus, Penicillium species,* and *Candida albican*, which form molds and yeasts fungi were the most common contaminants in our study is comparable with [15, 31] in New Dehli, India who reported *Aspergillus species*, *Penicillium*, and *Candida albican* as contaminants fungi. A possible explanation is that *Aspergillus species* and *Candida albicans* are known respiratory pathogens and can occur as co-infections in patients with *Mycobacterium tuberculosis*. In this study,72% of *Staphylococcus species* isolates show susceptibility to Ciprofloxacin, Gentamicin, and Chloramphenicol, Trimethoprim (57%) while was resistant to Erythromycin (72%) and Tetracycline (100%). This finding is compared to that of [32-34] in Nigeria, Mbarara Regional Referral Hospital, and Ethiopia, which found *Staphylococcus aureus* are sensitive to gentamycin, and ciprofloxacin (68.63%, 66.67%), (76.2%,71.4), and (100%). This may be due to their low prescription rates, high cost, and less frequent use due to intravenous or intramuscular administration hence less abused compared to oral antibiotics and side effects of some drugs. Another study in Algeria conducted by [35] revealed that *S. aureus* was resistant to ciprofloxacin, Tetracycline, and Gentamicin, respectively; however, in this study, it has been found that both the ciprofloxacin and Gentamicin, were highly sensitive to *S. aureus* except for tetracycline being resistant. This discrepancy can be explained by geographic variations and different antibiotic usage policies, leading to selective pressure and the emergence of resistance. Particularly *Staphylococcus aureus* exhibits resistance to tetracycline primarily due to the presence of specific genes like tetK and tetM that mediate this resistance, these genes encode efflux pumps and ribosomal protection mechanisms, making the bacteria resistant to tetracycline and related antibiotics [36].*S. epidermidis* was sensitive to gentamicin, and ciprofloxacin (50%) while resistant to trimethoprim, erythromycin, and chloramphenicol (100%). Our results are comparable with resistance observed in an Iran study that revealed *Staphylococcus epidermidis* resistance to tetracycline (97.30%), erythromycin (82.60%), and trimethoprim-sulfamethoxazole (73.91) [37]. This pattern points to the fact that the common presence of resistance mechanisms within *S. epidermidis*, is potentially due to biofilm

formation and horizontal gene transfer, which are well-documented in this species [38].*Streptococcus viridans* indicated that 100% of the isolates were susceptible to chloramphenicol and vancomycin while it was resistant to Trimethoprim (100%). This was comparable with the report by [39] in Uganda revealed that *Streptococcus viridans* is (100%) sensitive to Vancomycin, ceftriaxone (96%), and Clindamycin (81.3%) and also in contrast to Nepal shows the tetracycline (86.33%) was the most effective drugs to *Streptococcus species* [40]. Furthermore, because *viridans group streptococci* (VGS) have historically been disregarded in clinical settings and thought to be commensal flora, susceptibility testing for these bacteria is frequently not carried out regularly, and this customary carelessness leads to gaps in our knowledge regarding their resistance characteristics. *Escherichia coli* revealed a higher rate of resistance to ciprofloxacin (100%), this agrees with the finding of [41, 42] in Uganda and Ethiopia, in which ciprofloxacin (90%) Nalidixic acid (90%), and trimethoprim-sulfamethoxazole (33%) were ineffective to *E.coli*, this can be possibly due to ciprofloxacin has always being used in the empirical treatment in hospitals sitting. Itraconazole susceptible (MIC ≤ 0.12µg/ml) was observed in 83% of *Candida albicans*, while amphotericin B susceptible (MIC of ≤0.5 µg/ml) was observed in 66.7% of the same isolates. This finding aligns with the results reported in Kenya regarding the susceptibility of *Candida albicans* to itraconazole (92%), voriconazole (71%), and fluconazole (100%) [15] This suggests that resistance to these antifungal agents is uncommon in yeast isolates and also the limited availability of triazole. Caspofungin and 5-flucytosine resistance (MIC ≥1µg/ml, and ≥4µg/ml) was 33% and 50% in *Candida albican* respectively, but this is non-comparable with [43] in Arua Regional Referral Hospital West Nile Region of Uganda, report 97% and 100% of *Candida albicans* susceptibility to flucytosine and caspofungin. The VITEK ®2 compact system used by Ocan *et al* might have a different range of antifungal concentrations compared to those used in manual microdilution tests, this difference could lead to variations in the detected MICs and insufficient incubation time [44]. Additionally, itraconazole, amphotericin B susceptible (MIC <0.5g/ml) was observed in 100% for both *non-albican candida* and *Cryptococcus neoformans* (MIC (0.25g/ml), and the 5-flucytosine susceptible-dose dependent (≥8µg/ml) was seen in *Cryptococcus Neoformans*. This finding is also non-comparable with the study by [45, 46] in Mbale Regional Hospital, Eastern Uganda and Burkina Faso reported itraconazole, amphotericin B, and fluconazole were the most ineffective agents against *Candida albicans* and *non-albican candida*. Variations in Laboratory procedures could explain these, we employed the microdilution method which is the standard for determining MIC values, while their study used the disc diffusion method. For Itraconazole and Amphotericin B, both *Aspergillus flavus* and *Penicillium species* exhibited complete susceptibility, with MIC values of ≥1 µg/ml and ≥2 µg/ml, respectively. A comparable study conducted in Mbarara [21] Revealed that molds exhibited 97.5% and 70% sensitivities to Itraconazole and Amphotericin B, respectively and this suggests that Itraconazole is a fungistatic antifungal agent that alters membrane fluidity by inhibiting 14-

sterol demethylase. On the other hand, Amphotericin B is a fungicidal agent that attaches itself to ergosterol in the fungal cell membrane, causing holes to form, ion leakage, and fungal cell death [47]. *Aspergillus flavus* exhibited higher resistance to caspofungin with an MIC of ≥2 µg/ml, while *Penicillium species* were 100% susceptible, with MICs at <0.5 µg/ml. This is due to a potential involvement of P-type ATPase and ubiquinone biosynthetic methyltransferase COQ5 in the clinical isolates of *Aspergillus flavus* resistant to Caspofungin [48].Furthermore, both *Aspergillus flavus* and *Penicillium species* showed a MIC of 0.25 µg/ml for 5-Flucytosine, indicating uniform susceptibility to this antifungal agent. According to [49], 5-Flucytosine has little inherent activity against molds, this is due to the absence or low prevalence of the key resistance mechanisms in these particular fungal genera. To our knowledge, there are limited published studies available that comprehensively address the susceptibility of filamentous fungi.

5. CONCLUSION

Contamination of TB sputum cultures was observed in patients with suspected TB in this study. Our study found that bacteria and fungi contribute to TB sputum culture contamination the most common bacteria were, *S. aureus, Bacillus subtilis*, and *E. coli* was the least common, While *Candida species* and two filamentous fungi were the predominant fungal isolates in contaminated culture. The bacteria isolates were sensitive to Vancomycin, Gentamycin, and chloramphenicol and both yeasts and Molds were sensitive to Itraconazole, and Amphotericin B.

6. RECOMMENDATION

A Study to identify the sources of contamination, and *E. Coli* and *Aspergillus flavus* must be closely monitored since they may co-infect with tuberculosis (TB), which might make treating these infections more difficult, especially in those with weakened immune systems. Routine culture and drug susceptibility for Fungi and bacteria pathogens in all the suspected TB cases to avoid misdiagnosed and needless use of antimicrobial chemotherapy.

ETHICAL APPROVAL

Ethical clearance was received from the Faculty Research Committee of Mbarara University of Science and Technology.

Consent

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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