Original Research Article

Sputum Culture Contaminants and their Drugs Susceptibility Patterns among TB-suspected Patients at Epicentre Mbarara Research Centre, Uganda

ABSTRACT

Aims: This study aimed to determine the prevalence of sputum culture contamination, characterization, and drug susceptibility patterns among 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 Oxygen and Carbon dioxide incubators, with growth observed within 24 to 48 hours for bacterial contaminants and up to three days for yeast contaminants. Sabouraud Dextrose Agar cultures were monitored for two weeks for filamentous fungal development. Antibiograms for bacterial isolates were determined using the Kirby-Bauer disc diffusion method, while broth microdilution was used for fungal isolates.
Results: Of 81 samples, 14 were contaminated, yielding a contamination rate of 17.28%. The most common bacterial contaminants were *Staphylococcus aureus* (57.1%), *Bacillus subtilis* (42.9%), *Streptococcus viridans* and *Staphylococcus epidermidis* (22.2%), and *Escherichia coli* (11.1%). Fungal contaminants included *Aspergillus flavus*, *Penicillium species* (50%), *Candida albicans* (75%), *Cryptococcus neoformans*, and *non-albican Candida species* (12.5%). 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: There is a high prevalence of contamination in TB sputum cultures, highlighting the need for stringent aseptic techniques alongside current standards to improve the detection of *Mycobacterium tuberculosis*.

Keywords: sputum culture contamination, drug susceptibility patterns, Bacterial contaminants, Fungal contaminants

1. INTRODUCTION

Mycobacterium tuberculosis is a member of the slow-growing mycobacteria group that causes TB [1]. Globally, an estimated 10.0 million individuals worldwide are expected to be afflicted with tuberculosis, and Africa bears a disproportionately high burden of TB, with 23% of cases of dually infected people living on the continent [2].In Uganda, TB infection is highly endemic, ranked 16th among the 22 countries with the highest global TB burden, in 2018,

approximately 4000 new cases were diagnosed, and a high mortality rate has been reported [3, 4]. The difficulties associated with TB diagnosis continue to be a major impediment to combating the disease [5]. Despite its low sensitivity, sputum microscopy is frequently used as a primary diagnostic method in resource-limited settings [6]. More automated molecular tools such as Xpert MTB/RIF and the urine-based Lipoarabinomannan (LAM) test are being used for rapid diagnosis; however, their use is fraught with complications, the TB LAM test is still suboptimal, and the Xpert MTB/RIF assay is expensive, furthermore the Xpert MTB/RIF assay cannot reliably distinguish between passive and active infection, which is a significant clinical limitation, *Mycobacteria* cultivation, on the other hand, is the only technique for distinguishing between bacterial cells that are alive and deceased [7]. This aspect was often recruited to monitor treatment responses, confirm sterility of the patient and conversion of sputum, break transmission cycles, and document patient cure [8]. In addition, culture methods have demonstrated high TB diagnostic sensitivity and specificity, making them the gold-standard diagnostic approach [9]. Furthermore, using liquid culture (Mycobacterium Growth Indicator Tube) increases the number of bacteriologically confirmed tuberculosis cases by 20% - 30% [10]. Despite this, the tuberculosis culture is not widely available due to the high cost and laboratory biosafety infrastructure requirements [7]. Furthermore, the TB culture method remains suboptimal due to contamination issues, which impede mycobacterium isolation despite existing laboratory decontamination procedures [11]. The reliability of technical manipulation of analytical and pre-analytical processes impacts the likelihood of mycobacterial culture contamination, either positively or negatively [12]. Furthermore, soft 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 bacteria and fungi such as *S. pyogens, S. aureus, E. coli, K. pneumoniae, P. aeruginosa A. fumigatus,* and *C. albican* decreases the proportion of interpretable results and the diagnostic utility of culture systems [13]. 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. The etiology of sputum culture contamination was 40% in Burkina Faso [14].In Uganda, a 31%, and 15% contamination rate was reported in TB cultures [15, 16], while in Algeria 8.86% contamination was observed [17]. Tuberculosis sputum culture contamination is caused by various microorganisms, including bacteria and fungi, that survive the lab pre-culture decontaminated method and some may be resistant to currently available antibiotics and antifungal agents [14]. Unfortunately, little is known about the specific contaminants and drug susceptibility patterns of the isolated contaminants at the Epicentre Mbarara Research Centre TB laboratory. The following

antibiotics such as vancomycin 30µg, nalidixic acid 30µg, trimethoprim 5µg, and polymyxin B 300Iµg were used to test against bacterial contaminants and found nalidixic acid is incapable of prohibiting the development of the majority of the contaminants [18]. Furthermore, 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 [19]

$n = Z^2 P (1 – P) / d^2$

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 [13] 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 [20]

$n = \frac{n_0}{1+n_0/N} = \frac{143}{(1+143)/75} = 74$ 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 [13]. Liquid culture using Mycobacterium Growth Indicator Tube (MGIT) system is a widely used method for the detection and identification of *Mycobacterium tuberculosis* complex (MTBC) from all clinical specimens except blood [21]. The processed samples were inoculated into MGIT tubes containing modified Middlebrook 7H9 broth, supplemented Polymyxin B Amphotericin B

Nalidixic acid Trimethoprim Azilocillin to inhibit the growth of contaminants, and incubated in an automated BACTEC MGIT 960 system, which detects the presence or absent of organism by monitoring oxygen depletion in the culture medium [16]. When an organism is present, it metabolizes the oxygen in the medium, triggering a fluorescent signal that is detected by the instrument. Positive MGIT culture indicates the presence of Mycobacteria, however, further identification is required to distinguish MTBC from non-tuberculous mycobacteria (NTM), this is typically done using smear and stained with Ziehl Neelsen and the Gram smear to determine the possibility of other bacteria and their Gram reaction (either Gram-positive or negative). 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 identified. Solid cultures using Lowenstein Jensen slant is an egg-based culture medium that supports the growth of MTB as well as other mycobacterial species. Clinical processed specimens were, inoculated into LJ slope media containing malachite green, glycerol, and other nutrients that selectively promote the growth of mycobacteria, the inoculated tubes were placed in an incubator at 37^{o}C for up to 8 weeks. A positive LJ culture shows brown, granular colonies, raised, and creamy in appearance. This distinct colony morphology helps differentiate MTBC from other mycobacteria. Five of slopes contaminated LJ culture, where non-mycobacterial microorganisms grow has 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 [4].

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, per laboratory waste disposal protocols. These preliminary identification methods were conducted at the Microbiology unit of Epicentre Mbarara Research Centre Laboratory, which is located within the premises of Mbarara University of Science and Technology.

2.9 Culture and isolation of fungal contaminants

The cottony colonies from the contaminated LJ cultures were presumed to be contaminated with filamentous fungi and were inoculated on duplicated Sabouraud dextrose agar. The inoculated plates were incubated for two weeks at different temperatures to encourage

fungal growth, and they were checked every day to see if mold was growing at 20-30^{0}C and if the yeast was growing at 37^{0}C as previously described by [22]. Like LJ slant media, all positive tubes of MGIT presumed to be contaminated were also sub-cultured (10µl) onto Sabouraud dextrose agar and incubated for fungal growth. Viable yeast and mold cultural isolates were identified by observing colony morphology, Gram stain, Germ tube test, India Ink, CHROMagar media, and lactophenol cotton blue. After two weeks, 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. The culture elevation, consistency, rate of development, and coloration on both the front and back were noted [23]. 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 [24]. 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 identify 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, show Gram-positive yeast which are round to oval in shape and larger than bacteria, ranging from 3 to 4 µm in diameter [25]. These appearances are typical of *Candida species* and Germ tube formation and CHROMagar media was done to differentiate the *Candida albicans* and *Non-candida species,* and India Ink, urease test was used to identify *Cryptococcus neoformans* [26].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 previously described by [27]. A pure culture of the identified bacterial isolates was chosen from a 24-hour plate culture. Three colonies of each type of bacterial growth were selected using a sterile wire loop, then emulsified in five milliliters of sterile normal saline as previously described by [28]. After calibration, 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 reached. 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. Swab the entire surface area of the Mueller-Hinton agar plates completely by rotating the plate. Allow the plates to dry for five minutes so that the medium absorbs the inoculum properly before applying antibacterial drugs to the plates [29]. 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. Place sterile paper discs containing specific concentrations of various antibiotics onto the inoculated agar surface at a distance of 24mm using sterile forceps [30]. 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 Clinical and Laboratory Standards Institute [31]. Antifungal susceptibility testing was performed in compliance with the Clinical and Laboratory Standards Institute M27-A3 and M38-A2 [32, 33] on the 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 [24, 34]. 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, descriptive statistics in the form of frequencies and percentages were generated

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%), with the overall prevalence of sputum culture contamination was 17.28 % (95%CI: 10.4 - 22.22). Among the 16 bacterial contaminants,15 were Gram-positive while 1 was Gram-negative, *Staphylococcus aureus* was the most prevalent accounting for 4(57.1%), and the least common was *Escherichia coli* 1(11.1%) as shown in Table 1 below.

Table 2 showed eight yeast and two mold contaminants identified, with *Candida albicans* being the most prevalent, accounting for 6 (75%). *Cryptococcus neoformans* and *non-albicans Candida* were the least common, representing 1(12.5%). Among the filamentous fungi identified, both *Penicillium* species and *Aspergillus flavus* were detected, with 1 (50%). Table 3 showed 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. *Bacillus subtilis* 3(75%) of the isolates were susceptible to Tetracycline, while it resisted Erythromycin 3(75%). *Escherichia coli* showed high resistance to Ciprofloxacin (100%) but was 1 (100%) intermediate to the other four drugs.

Table 4 shows the susceptibility patterns of various antifungal agents against yeast and filamentous species showing that *Candid 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.

Table 1: The frequency of bacteria identified from contaminated MGIT and LJ slant medium

Contaminants	**No. of contaminants**	
	Bactec/Alert liquid media (% of total contaminants)	**LJ slope media (% of total contaminants)**
Staphylococcus aureus	4(57.1)	3(33.3)
Staphylococcus epidermidis	0(0)	2(22.2)
Bacillus subtilis	3(42.9)	1(11.1)
Streptococcus viridans	0(0)	2(22.2)
Escherichia coli	0(0)	1(11.1)
Total	**9(100)**	**7(100)**

The numbers in the bracket are percentages (%) of total bacterial contaminants per media

Table 2: The filamentous, yeast fungi and their macroscopic and microscopic characteristics

Yeast species	**Colony characteristics on SDA**	**CHROMagar**	**Identification by GTT**	**Identification by India Ink**	**No. of isolates n (%)**
C.albicans	White cream, smooth and yeast-like appearance	Green	Positive	*	6 (75)
C.neoformans	Gray	*	*	Positive	1 (12.5)

Non-candida albicans	Pasty, opaque, smooth, slightly domed, or flat and pale.	White pink	Negative	*	1 (12.5)
Total number of isolates					**8 (100)**

Filamentous fungi	**Colony characteristics on SDA (obverse)**	**Colony characteristics on SDA (reverse)**	**Structure of phialides identify by LPCB**	**No. of isolates n (%)**
Penicillium species	Bluish green, white margin, exudates present, raised with radial furrows	Creamish yellow	Branching, flash-shaped conidiophores with metulae	1(50)
Aspergillus flavus	Light green powdery colony	Cream-yellow	Biseriate phialides and aerial hyphae with rough, spiny conidiophores	1 (50)
Total number of isolates				**2 (100)**

*Keys: LPCB: Lacto phenol cotton blue, SDA: Sabouraud dextrose agar GTT: Germ tube test, *: no requirements*

Table 3: 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)
B. subtilis	4	S	2 (50)		2 (50)		1 (25)	3 (75)	
		R	2 (50)	*	2 (50)	*	3 (75)	1 (25)	*
		I	0 (0)		0 (0)		0 (0)	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 4: 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)

Filamentous isolates	No	Patterns	Antifungal agents tested			
			ITZ	AMB	CAS	FCY
			No (%)	No (%)	No (%)	No (%)
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 [35] in India reported a lower contamination rate of 5.6%, which might be attributed to the difference in the sample size of 800 compared to only small samples used in our study. Additionally, variations in the local environment, such as climate and microbial flora, may influence contamination rates. The specific environmental conditions in Mbarara could promote the growth of contaminants that are less prevalent in regions like India, potentially leading to higher contamination in this study.

However, our finding is comparable with contamination reported in Northern Ireland 10% [18], Tanzania 12% [36], Ethiopia 22 % [21] , Uganda 10.4% [13]. This agreement is due to similar culture media (LJ and MGIT) for sample processing. However, our contamination is lower than what was reported in studies conducted in Botswana 35.6% [37] , and Burkina Faso 40% [14]. According to Ho-Foster et al.,2018, the probable explanation for the difference may be due to the sample size and delays in transportation and the distance from the collection site to the laboratory which might have allowed more opportunities for contamination.

A study by Kabore *et al* reported that mouthwash with chlorhexidine did not considerably lower contamination, and sporulated bacteria were the major residual contaminants this suggests that the upstream reduction of oral microbial load does not induce the downstream reduction of culture contaminations. Additionally, the sporulating bacteria are more resistant to chemical substances including chlorhexidine used for mouthwash in their study or the concentration of antiseptic usage cannot eliminate the sporulating organisms.

Staphylococcus aureus, Staphylococcus epidermidis, *Bacillus subtilis, and Escherichia coli* were the most common bacterial contaminants found in this study. It has been demonstrated in South Asia, Nepal, that *Streptococcus spp* and *Pseudomonas aeruginosa* are one of the bacteria that form colonies in *Mycobacterium tuberculosis* cultures [38]. Which is strongly non-comparable with the results of this study. The environmental condition might be the most likely reason for the discrepancy and sample processing method. *Candida albicans, Cryptococcus neoformans, Penicillium species, and Aspergillus flavus* were the most prevalent fungal contaminants in our study and the most common bacterial contaminant was *Staphylococcus aureus*, it is similar to a study conducted in Northern Ireland, UK, which found that *Staphylococcus epidermidis* was the most frequently isolated contaminant [18].

The findings of this study, which identified Gram-negative and Gram-positive bacteria from LJ slants and MGIT cultures align with the results of [4] in Uganda, except for the absence of *Streptococcus pyogenes*. Additionally, [15, 39] reported *Staphylococcus* species in antibiotic-free LJ culture tubes from patients at Mbarara Regional Referral Hospital, and in a French hospital. *Escherichia coli* was among the least common contaminants from the finding, consistent with its reported presence as a major contaminant by Vianney et al. at the NTRL.

This suggests 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 that the concentration of antibiotics assumed to be effective against these

microorganisms (Trimethoprim and Nalidixic acid, Polymyxin B respectively), might not be effective in suppressing these organisms.

Except for *Staphylococcus, Bacillus*, and *Streptococcus species*, the bacteria contaminants isolated in this study varied from those recovered using pyrosequencing in another study [18]. The variations of the result were from the study of molecular-based identification methods with greater dynamic range, high sensitivity, and specificity.

Aspergillus flavus, Penicillium species, and *Candida albican*, which forms molds and yeasts fungi were the most common contaminants in our study is comparable with [24, 40] 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*. Our findings, however, cannot be compared to [41] in North East India, who found *Candida albican* as the common yeast without filamentous fungi. The differences can be the lack of Sabouraud Dextrose Agar for the isolation and identification of fungal contaminants in TB sputum cultures.

This study also provides information on the antibiotic susceptibility profiles of bacteria isolated from contaminated TB sputum cultures. The overall pattern of antimicrobial susceptibility of bacteria isolates in this study indicates that they have a resistance of 50%, and 73% against erythromycin and trimethoprim and this was in line with earlier research carried out in Tanzania [42], Unrestricted over-the-counter sales of antibiotics, mostly for self-treatment of human diseases without a prescription, maybe the cause of resistance and this would unavoidably result in the establishment and quick spread of resistant strains.

In our study, we found that gentamycin, vancomycin, chloramphenicol, and ciprofloxacin were effective against isolates, similar to Ethiopia's findings [43]. This may be due to their low prescription rates, high cost, and less frequent use due to intravenous or intramuscular administration like gentamycin hence less abused compared to oral antibiotics and side effects of some drugs. However, our finding is non-comparable with Malawi which found gentamycin and chloramphenicol were resistant against the isolates [44] This can be explained due to the geographical location.

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 slightly correlated to that of [45-47] 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%).The similarity was attributed to the same method applied. Another study in Algeria conducted by [48] 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 [49].

S. epidermidis was sensitive to gentamicin, and ciprofloxacin (50%) while resistant to trimethoprim, erythromycin, and chloramphenicol (100%). Our results are per resistance observed in an Iran study that revealed *Staphylococcus epidermidis* resistance to tetracycline (97.30%), erythromycin (82.60%), and trimethoprim-sulfamethoxazole (73.91) [50]. This pattern suggests the common presence of resistance mechanisms within *S. epidermidis*, potentially due to biofilm formation and horizontal gene transfer, which are well-documented in this species [51].

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 [52] 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* [38]. This could be due to less exposure to selective pressure. 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.

Bacillus species were predominantly sensitive to Tetracycline (75%) Vancomycin (50%) and Ciprofloxacin (50%), although showing resistance to erythromycin (75%). This is non-comparable to the study done in South Africa which showed tetracycline, and fluoroquinolones were resistant [53]. This was probably because variations in techniques and protocols can lead to differing results and interpretations. Erythromycin resistance in *Bacillus species* is often caused by genes that methylate the 23S rRNA at macrolide binding sites [54].

Escherichia coli revealed a higher rate of resistance to ciprofloxacin (100%), this agrees with the finding of [55, 56] in Uganda and Ethiopia, in which ciprofloxacin (90%) Nalidixic acid (90%), and trimethoprim-sulfamethoxazole (33%) were resistant to *E.coli*, this can be possibly due to ciprofloxacin has always being used in the empirical treatment in hospitals sitting.

In our study, 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%) [24] This suggests that resistance to these antifungal agents is uncommon in yeast isolates and also the similarity in methodology.

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 [57] 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 [58].

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 [59, 60] in Mbale Regional Hospital, Eastern Uganda and Burkino Faso reported itraconazole, amphotericin B, and fluconazole were the most resistant agents tested against *Candida albicans* and *non-albican candida*.

Variations in Laboratory procedures could explain these, we employed the microdilution method for determining MIC values, while their study used the disc diffusion method. Based on the existing investigation, amphotericin B, and itraconazole are efficient against yeast isolates. In our research, we found *Cryptococcus neoformans* to be sensitive to Amphotericin B, itraconazole, and caspofungin (100%) and susceptible-dose dependent to 5-flucytosine, consistent with findings from the China study [61]. This can be due to the identical testing methodologies used in both studies. However, it is essential to note that limited data exist on susceptibility testing on bacterial and fungal contaminants, highlighting the necessity for further research to better understand antimicrobial resistance patterns and guide appropriate treatment strategies. This study details the patterns of mold susceptibility to antifungals that were identified from contaminated TB sputum cultures. Antifungal drugs, such as 5-Flucytosine, Itraconazole, Amphotericin B, and Caspofungin, were used against *Penicillium* and *Aspergillus species*. 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.

This suggests that Itraconazole and Amphotericin B are effective against both species at these concentrations. A comparable study conducted in Mbarara [34] Revealed that molds

exhibited 97.5% and 70% sensitivities to Itraconazole and Amphotericin B, respectively and this suggests that resistance to these antifungal agents is uncommon in mold isolates.

Caspofungin showed significant variability in MICs between the two fungal species. *Aspergillus flavus* exhibited higher resistance with an MIC of ≥2 µg/ml, while *Penicillium species* were 100% susceptible, with MICs at <0.5 µg/ml. Similar results in Iran indicated a potential involvement of P-type ATPase and ubiquinone biosynthetic methyltransferase COQ5 in the clinical isolates of *Aspergillus flavus* that are resistant to Caspofungin [62].

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 [63], 5-Flucytosine has little inherent activity against molds, corroborating the findings of this study and this indicates 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*, and *E. coli* was the least common, While *Candida species* 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.

ETHICAL APPROVAL

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

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