Evaluation of a Laboratory-Prepared Desoxycholate Medium for the Primary Isolation of Uropathogens

Kadafa, Adati Ayuba[1], Othman, Fadilah.[2]

1. Department of Environmental Management, Faculty of Environmental Studies, Universiti Putra Malaysia
2. Department of Environmental Management, Faculty of Environmental Studies, Universiti Putra Malaysia
kwaala@yahoo.com, fadhilah.env@gmail.com

Abstract: Urinary tract infection (UTI) is one of the most common bacterial infections seen in primary care. Performing urine cultures for the diagnosis of UTI is a significant part of the workload of most clinical microbiology laboratories. A large laboratory may examine 200-300 urine samples each day. This heavy work load reflects the frequency of UTI both in general practice and in hospital settings. MacConkey agar is a very popular medium for the primary plating of urine specimens. It is rather high cost and not so long shelf-life in the tropics has made it difficult for many laboratories to undertake routine cultures of urine specimens. It has therefore become necessary to attempt the formulation of a laboratory prepared substitute using basic chemical ingredients with long shelf-life so as to provide a substitute for the costly MacConkey agar. In this study thirty-two turbid urine samples that were routinely submitted by patients to the bacteriology diagnostic laboratory of Jama'a Hospital Samaru, Zaria, Nigeria were collected and streaked on plates of laboratory prepared Desoxycholate agar and commercial MacConkey agar using a calibrated wire loop able to deliver 0.01ml of fresh urine. The two media were then evaluated on the basis of bacterial growth promoting ability of each medium. Each plate was scored on the basis of exuberance of growth and colonial morphology of the urinary isolates. The plates were read for significance after incubation for 24 hours at $36^oC \pm 1^oC$. A plate was recorded as exhibiting significant bacteriuria when 0.01ml of fresh urine yielded confluent growth or near-confluent growth or indicated a number of colonies equal to or greater than one thousand (≥1000Cfu/0.01ml). Non-significant bacteriuria was recorded when the colonies were less than 1000 per 0.01ml of fresh urine (<1000Cfu/0.01ml). With regards to colony size and fermentation of lactose, the results showed the urinary isolates on both media were substantially the same. The results also showed that if the commercial MacConkey medium used as a standard reference in this evaluation were employed in the diagnostic laboratory, a small number of patients would be diagnosed falsely as having insignificant bacteriuria as a result of inadequate growth of organisms on the rather aged commercial MacConkey medium used in this evaluation. The result of this evaluation seems to indicate that the laboratory prepared Desoxycholate medium may be a reliable and much cheaper medium for the primary isolation of uropathogens in urine specimens. More extensive evaluation is required to validate these preliminary findings.

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1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections seen in primary care (Ishikawa et al., 2011; Medina-Bombardo and Jover-Palmer, 2011). Uropathogen; are the causative organisms of UTIs. Uropathogens include: *E.coli, Enterococcus sp., Streptococci sp., Proteus mirabilis, Pseudomonas sp., Staphylococcus sp., Citrobacter freundii*, *E. cloacae* , *and Morganella morganii* (Pezzlo, 1988; Scarparo et al., 2002; Halender and Dahl, 2005; Zorc et al., 2005; Ciragil et al., 2006; Ishikawa et al., 2011). *E. coli* is the most common bacterium isolated in clinical laboratories and is also the predominant pathogen (80%) in urinary tract infections; the primary causative (Pezzlo, 1988; Halender and Dahl, 2005; Ishikawa et al., 2011)

Commercial media for bacterial culture is often a risk to have; due to alteration quality compared to traditional prepared media. Several studies have been conducted to evaluate the comparison between commercial media and reference media for isolation and identification of UTI pathogen (Halender and Dahl, 2005; Aspevall et al., 2002; Ciragil et al., 2006). According to Ciragil et al. (2006), among the benefit of commercial media are the ability to detect and identify the pathogens that are frequently observed in UTI, save the cost by reducing the number of media used for primary isolation to only one, prevents the swarming of *Proteus sp.* thus enables correct quantitative evaluation and faster detection of the isolates, especially in mixed cultures. Despite that, there is a risk that false result will be obtained using this commercial media because of the alteration of few parameters.

The aim of the microbiology laboratory in the management of UTI is to reduce morbidity and

mortality through accurate and timely diagnosis of UTI and appropriate antimicrobial sensitivity testing of uropathogens. Performing urine cultures for the diagnosis of UTI is a significant part of the workload of most clinical microbiology laboratories (Scarparo et al., 2002; Ciragil et al., 2006). A large laboratory may examine 200-300 urine samples each day. This heavy work load reflects the frequency of UTI both in general practice and in hospital settings. In children, infection is more common in young girls, except in the neonatal age group, where boys predominate (Pezzlo, 1988; Zorc et al., 2005). It is estimated that 20% of women develop a UTI during their life time; the incidence increases at puberty and remains high throughout adult life, only after the age of 50 years is a similar incidence seen in males. UTI accounts for approximately 23% of all hospital acquired infections (Franz and Horl, 1999; Larcombe, 1999; Graham and Galloway, 2001).

Although the incidence of urinary symptoms is high, most specimens received from patients complaining of infection are negative samples (Stapleton, 1999). Most infections at all ages are the result of faecal enteric bacteria especially *Escherichia coli*, which colonise the perineum and then ascend the urethra to multiply and infect the bladder, kidney, and adjacent structures (Pezzlo, 1988; Zorc et al., 2005). The most common site of infection is the bladder (Ross and Kay, 1999; Delzell and Lefevre, 2000; Thomas, 2003; Kucheria et al., 2005; Colgan et al., 2006; Egland and Egland, 2006). Haematogenous infection of the urinary tract occurs most notably with *Mycobacterium tuberculosis* and *Salmonella sp*. and direct introduction of organisms during instrumentation of the urinary tract also is well recognised. Structural and functional abnormalities result in a wide range of possible infecting organisms (Sadovsky, 1998; Knowles, 2005). Urinary tract infection may occur with or without symptoms; the latter is known as covert or asymptomatic bacteriuria (Pezzlo, 1988; Finnell et al., 2011). Because urine must pass through the distal urethra and in women over the perineum, it may become contaminated by the normal flora of these regions (Mohsin and Siddiqui, 2010). Isolation of more than one bacteria strain suggests such contamination, but when a single strain is isolated, quantitative culture is required to determine whether it indicates true bacteriuria or it reflects mere contamination (Graham and Galloway, 2001; Colgan et al., 2006).

Traditionally, the cut-off point for significant bacteriuria is the presence of 10^5 organisms/ml of fresh urine (Barbin et al., 1978; Murray and Niles, 1981; Pezzlo, 1988; Aspevall et al., 2002; Scarparo et al., 2002; Halender and Dahl, 2005; Zorc et al., 2005; Ishikawa et al., 2011). Recently, this cut-off point has been revised downwards so that 10^3 to 10^4 organisms/ml of fresh urine can be acceptable to define significant bacteruria provided other indicators of infection such as monomicrobic infection, pyuria, haematuria and clinical signs of UTI are present (Pezzlo, 1988; Franz and Horl, 1999; Larcombe, 1999; Graham and Galloway, 2001; Sadovsky, 2001; Halender and Dahl, 2005; Colgan et al., 2006). These cut-off values can be applied to all rapidly growing bacteria but not fungi or fastidious organisms. The detection of polymorphonuclear cells (pyuria) and red blood cells (haematuria) in urine is useful for the diagnosis of infection or other renal tract pathogens (Kucheria et al., 2005).

Research funded by the National Institute of Health (NIH) suggests that one factor behind recurrent UTIs may be the ability of bacteria to attach to cells lining the urinary tract. A recent NIH funded study found that bacteria formed a protective film on the inner lining of the bladder in mice. If a similar process can be demonstrated in humans the discovery may lead to new treatments to prevent recurrent UTIs (Daifuku and Stamm 1986). Another research has indicated that women who are “non-secretors” of certain blood group antigens may be more prone to recurrent UTIs because the cell lining the vagina and urethra may allow bacteria to attach easily.

2. Overview on Urinary Tract Infection

Bacterial infections of the urinary tract are the second most common type of infection in the body. Urinary infection accounts for about 8.3 million doctor visits each year in the United States of America (Gaspari and Bosker, 2003; Burt and Schappert, 2004; Mehnert-Kay, 2005; Ishikawa et al., 2011). Infections are relatively rare in younger men, but they are common problems for men older than 50 years (Pezzlo, 1988; Graham and Galloway, 2001; Manges et al., 2001; Jones, 2002; Thomas, 2003). One out of every five women develops urinary tract infection (UTI) in their life time (Smith and Brumfitt, 1984). Urinary tract infections in men are not as common as in women but can be very serious when they do occur (Pezzlo, 1988; Hooton et al., 1996; Kucheria et al., 2005).

Urinary tract infection can often be classified into two types based on their location in the urinary tract. Lower tract infections include cystitis; bladder infection and urethritis; infection of the urethra (Pace, 2000; Zorc et al., 2005). Lower tract infections commonly are caused by bacteria, which enter and contaminate the urinary tract from the rectum via the urethral orifice. Urethritis may also be caused by microorganisms such as *Neisseria gonorrhoea* and *Chlamydia trachomatus* through

sexual contact (Graham and Galloway, 2001). Another form of male urinary infection is prostatitis, which is an inflammation of the prostate (Lipsk et al., 1999; Mims et al., 1990; Thomas, 2003; Varshney, 2003). Meanwhile upper tracti involves the ureters and the kidneys and it is referred to as pyelonephritis; kidney infection (Pace, 2000). Upper tract infection often occurs because bacteria ascend in the urinary tract from the bladder to the blood stream and have reached the kidney to set up infection (Gauser, 1981).

A number of factors contribute to urinary tract infection. Any abnormality of the urinary tract that obstructs the flow of urine such as kidney stone sets the stage for an infection. An enlarged prostate gland also can slow the flow of urine, thus raising the risk of infection. A person who cannot void or who is unconscious or critically ill often needs a catheter that stays in place for a long time. Some people, especially the elderly or those with nervous system disorder who lose bladder control may need a catheter for life and bacteria on the catheter can infect the bladder (Pezzlo, 1988; Donovan, et al., 1977; Mohsin and Siddiqui, 2010). People with diabetes have a higher risk of UTI because of lowered immunity associated with diabetes (Pezzlo, 1988; Mohsin and Siddiqui, 2010). Any other disorder that suppresses the immune system raises the risk of a urinary infection (Mohsin and Siddiqui, 2010).

UTI may occur in infants, both boys and girls, who are born with abnormalities of the urinary tract (Pezzlo, 1988; Bello, 1988). Such abnormalities of the urinary tract sometimes require surgical correction and are very common in infant boys (Bello, 1988; NKUDIC, 2005). There is clearly a higher frequency of UTI in women compared to men in the prime of life (Pezzlo, 1988). One factor may be that a woman's urethral opening is in close proximity to the anus from which bacteria contaminate the vagina (Jones, 2002; Knowles, 2005; Hooton and Stamm, 2006; Mohsin and Siddiqui, 2010). For many women, sexual intercourse seems to increase the frequency of infection, because sexual intercourse enhances entry of bacteria into the bladder via the urethral meatus (Wong and Stamm, 1983; Thomas, 2003; NKUDIC, 2004; Knowles, 2005; Mehnert-Kay, 2005; Hooton and Stamm, 2006).

Pregnant women seem no more prone to UTIs than other women (Mohsin and Siddiqui, 2010). However, when UTI does occur in a pregnant woman, it is usually likely to travel to the kidneys. According to some reports, about 2 to 4 percent of pregnant women develop a urinary tract infection. Scientists think that hormonal changes and shift in the position of the urinary tract during pregnancy makes it easier for bacteria to travel up the ureters to the kidneys for this reason, doctors recommend periodic testing of urine during pregnancy (Smith and Brumfitt, 1984; Delzell and Lefebvre, 2000).

Due to the frequency in occurrence of UTI and submission of urine specimen in the bacteriology clinical laboratory, processing of urine specimens is responsible for a large portion of the running cost of a laboratory. A substantial reduction in the cost of processing urine specimens will affect substantial savings in the running cost of a diagnostic laboratory.

MacConkey agar is a very popular medium for the primary plating of urine specimens. It is rather high cost and not so long shelf-life in the tropics has made it difficult for many laboratories to undertake routine cultures of urine specimens. It has therefore become necessary to attempt the formulation of a laboratory prepared substitute using basic chemical ingredients with long shelf-life so as to provide a substitute for the costly MacConkey agar. A preliminary evaluation of such a medium had been carried out some years back and some defects were noted by Uzuative (1995). The present exercise is an evaluation of a new MacConkey substitute with more reliable performance based on a revised formula. The cost of making a unit of the Desoxycholate medium from basic chemical ingredients is expected to be far less than the cost of a unit of the commercial MacConkey agar, basically because the ingredients are cheaper to procure in bulk and store longer under tropical conditions than the commercial MacConkey medium.

The purpose of this work is to compare the efficiency of laboratory prepared Desoxycholate medium with that of the commercial MacConkey medium in the primary isolation of pathogens associated with urinary tract infections.

2.1 Epidemiology

UTIs are common in general practice accounting for 1-3% of all consultations. The frequency of acute cystitis in young women is of the order of 0.5-0.7 episodes per year and approximately 25% of these will develop recurrent episodes. UTIs occur much less frequently in men at all ages (Pezzlo, 1988). Patients of either sex are more likely to develop a UTI if there is an abnormality of the renal tract or if there has been recent instrumentation of the renal tract. Antibiotic use changes the vaginal flora and promotes colonisation of the genital tract with *E. coli* resulting in subsequent increased risk of UTI. Other risk factors associated with UTI include recent sexual activity, new sexual partner and use of spermicide. There may also be a genetic component to risk as there is an increased incidence of UTI in the immediate female relatives of women with recurrent UTI; this theory is also supported by the fact that UTIs are 3-4 times more likely to occur in women

certain blood groups (Hooton et al., 1996; Stapleton, 1999). Other influencing factors of UTI is listed in Table 1.

painful, burning feeling in the area of the bladder or urethra during urination. It is not unusual to feel erect, shaky, washed out and to feel pain even when not urinating. Often women feel an uncomfortable pressure above the pubic bone, and some men experience fullness in the rectum. It is common for a person with a urinary infection to complain that, despite the urge to urinate; only a small amount of urine is passed. The urine itself may look milky or cloudy, even reddish if blood is present. Normally, UTI does not cause fever if it limited to the bladder or urethra. A fever may mean that the infection has reached the kidney. Symptoms of a kidney infection include pain in the back or side below the ribs, Fever, nausea or vomiting, polyuria, dysuria, burning, and frequency of micturition have been noted in patients.

2.2 Symptoms of Urinary Tract Infections

Not everyone with a UTI has symptoms, but most people experience at least some symptoms. These may include a frequent urge to urinate, a

(Jeena et al., 1996; NIH, 1999; Ross and Kay, 1999; Pace, 2000; Bagga, 2001; Jones, 2002; Knowles, 2005; Zorc et al., 2005; Ishikawa et al., 2011). Nitrituriais is clearly the most useful diagnostic indicator (Medina-Bombardo and Jover-Palmer, 2011). In children UTI is difficult to detect, symptoms of a urinary infection may be over-noised or attributed to another disorder (Jeena et al., 1996). A UTI should be considered when a child or infant seem irritable, or is not eating normally, has an unexplained fever that does not go away, has incontinence or loose bowel or is not thriving (Ross and Kay, 1999; NIH, 1999; Bagga, 2001; Musa-Aisien et al., 2003; Egland and Egland, 2006). Other influencing factors if UTI is listed in Table 1.

Table 1: Influencing factors of UTI

Mortality/Morbidity	Mortality related to acute UTI is exceedingly rare for otherwise healthy individuals in developed countries. UTI is a common paediatric problem with the potential to produce long-term morbidity (Zorc et al., 2005). Morbidity associated with pyelonephritis is due to systemic symptoms, such as fever, abdominal pain, vomiting and dehydration. Bacteriuria and clinical sepsis may occur. Individuals with acute pyelonephritis also may have acute cystitis. Long term complications of pyelonephritis are hypertension, impaired kidney function, end-stage renal disease (ESRD) and complications of pregnancy (Bircan, 2002; Egland and Egland, 2006; Mehnert-kay, 2005; Hellerstein, 2006). Bacterial entry into the blood stream is associated with severe morbidity, including sepsis and death (Mohsin and Siddiqui, 2010).
Race	Data are scant; however one study by Hoberman et al., (1993) showed prevalence of a febrile UTI in infants exceeding that in black infants.
Sex	During the early ages of life male incidence exceeds that of females. But in the prime of life both first time and recurrent UTIs is much more common in females (Pezzlo, 1988).
Age	Most first time UTIs is common in the first 2 years of life for children, men that are elderly and middle aged women (Jeena et al., 1996).

2.3 Factors predisposing to UTI

For patients who receive broad-spectrum antibiotics (e.g. amoxicillin, cephalexin), which are likely to alter gastro intestinal (GI)and periurethral flora, are at increased risk because of disturbance of the natural defence against colonisation by pathogenic bacteria.

Prolonged incubation of bacteria in bladder urine due to incomplete bladder emptying or infrequent voiding may compromise an important bladder defence against infection. Symptoms of voiding dysfunction such as, urgency, frequency, hesitancy, dribbling, or incontinence may occur in the absence of infection or local irritation because of uninhibited detrusor contractions. When incontinence is prevented by obstruction of the urethra, milking back of bacteria-laden urine may occur from the distal urethra into the urinary bladder. This mode of bacterial access is a common predisposing factor to UTI among paediatric patients.

Voiding dysfunction is usually not encouraged until a child is in the process of achieving daytime urinary control. Children with voiding dysfunction may attempt to prevent incontinence during uninhibited detrusor con traction by voluntarily increasing outlet resistance. This may be achieved using various posturing manoeuvres, such as tightening of the pelvic floor muscles or applying direct pressure to the urethra (e.g. dancing, squatting, Vincent Curtsy).

Constipation, with the rectum chronically dilated by faeces, is another important cause of voiding dysfunction. Finally neurogenic or anatomical abnormalities for voiding dysfunction can exist. Neonatal circumcision decreases the risk of UTI by about 90% in male infants during the first year of life. The risk of UTI in a circumcised infant during the first year is about 1 in 1000, while an uncircumcised infant has a 1 in 100 chance of developing a UTI (Hellerstein, 1998).

2.4 Diagnosis

Diagnosis is based on quantitative cultures of a properly collected urine specimen. A midstream clean-catch specimen may be obtained from children who have acquired urinary control. In the infant or child unable to void upon request, obtain the specimen for culture by suprapubic aspiration or urethral catheterization (Zorc et al., 2005). Suprapubic aspiration is the method of choice for obtaining urine from the uncircumcised male whom the urethral meatus cannot be observed without causing discomfort and in children of either sex with significant periurethral irritation. Culture of a urinary specimen obtained by collection in a sterile bag attached to the perineal area, which shows no growth or very scant growth (<10,000 colony-forming units [Cfu]/mL), is strong evidence of the absence of UTI (Pezzlo, 1988; Scarparo et al., 2002; Medina-Bombardo and Jover-Palmer, 2011). Unfortunately the false-positive rate is so high that this method of urine collection is not suitable for diagnosis. Urinalysis cannot substitute for urine culture to document the presence of UTI; however, it can help to identify children who should receive antibacterial treatment while awaiting culture results of a properly collected specimen of urine (Ross and Kay, 1999; Roberts, 2000; Graham and Galloway, 2001; Thomas, 2003). For children with a presumptive diagnosis of acute pyelonephritis obtain a CBC count and basic metabolic panel for children with a presumptive diagnosis of acute pyelonephritis must be obtained. A blood culture for febrile infants and for older patients who are clinically ill, toxic, or have high fever must be carried out.

To identify an isolate, it must first be recovered from a specimen on a suitable primary medium. Blood agar, chocolate agar, CLED and MacConkey continue to be popular in many diagnostic laboratories (Murray and Niles, 1981; Aspevall et al., 2002; Scarparo et al., 2002). Their satisfactory performance over the years has contributed to the lack of effort of developing equally efficacious or better alternatives.

In developing countries however, the extremely high cost of MacConkey agar which is about the most popular primary isolation medium is beginning to generate high interest in the sourcing of cheaper alternatives. The present exercise is an evaluation of one such alternative that has been developed for primary isolation of uropathogens in urine specimen.

2.5 Treatment

UTIs are treated with antibacterial drugs (Zorc et al., 2005; Mohsin and Siddiqui, 2010; Finnell et al., 2011; Ishikawa et al., 2011). The choice of drug and length of treatment depend on the patient's history and the urine tests that identify the offending bacteria. Sensitivity is also useful in helping the doctor to select the drug of choice.

The typical drugs of choice are: trimethoprim-sulfamethoxazole (Bactrim®), nitrofurantoin (macrobid ®), ciprofloxin (cipro®) or levofloxacin (levaquin®). Most experts agree that trimethoprim-sulfamethoxazole (TMP.SMX) is the drug of choice for cystitis infection, unless there is known resistance to this drug, the patient is allergic to it or the patient is in the late stages of pregnancy. Cipro® and levaquin® are fluoroquinolones, which cannot be prescribed in pregnant women or nursing mothers (Franz and Horl, 1999; Delzell and Lefevre, 2000; Car and Sheikh, 2003; Gaspari and Bosker, 2003; NKUDIC, 2004; Zorc et al., 2005; Hooton and Stamm, 2006).

Symptoms generally resolved one to three days after starting treatment, but it is important to complete full course of antibiotics prescribed so the infections is completely eradicated. If needed medicine for pain relieving can be given for pains associated with the infection. Increasing fluid intake is recommended to help flush the bacteria from the bladder. There are also definitive studies on the effectiveness of cranberry juice for the treatment of a UTI.

Medina-Bombardo and Jover-Palmer (2011) wrote a review focused on studies that examined the diagnostic accuracy of at least one symptom, sign or patient antecedent related to the urinary tract. Included where urine culture, a gold standard, was performed by primary care providers on female subjects aged at least 14 years. A meta-analysis of the likelihood ratio was performed to assess variables related to the urinary tract symptoms. There study concluded that Clinical findings do not aid in the diagnosis of UTI among women who present with urinary symptoms. Vaginal discharge is a weak indicator of the absence of infection. The urine dipstick test was the most reliable tool for detecting UTI.

3. Materials and Methods

Thirty-two turbid urine specimens routinely submitted by patients to the bacteriology diagnostic laboratory at Jama'a. Hospital Samaru, Zaria were used in evaluating the laboratory prepared Desoxycholate agar.

The urine specimens were collected in sterile plastic universal containers and transported quickly to the laboratory for processing (Ciragil et al., 2006). When the urine specimens could not be processed within one hour of collection, they were refrigerated at 4 – 8°C for a few hours until they could be processed (Murray and Niles, 1981; Aspevall et al., 2002; Scarparo et al., 2002). Commercial MacConkey agar was prepared from stock powder following manufacturer's instructions. The laboratory prepared Desoxycholate agar, was prepared using the laboratory derived formula and following laboratory instructions.

Each urine specimen was inoculated of the commercial medium and the laboratory prepared medium using calibrated wire loop which delivered 0.01ml of urine (Barbin et al., 1978). The 0.01ml inoculum was delivered unto the medium and spread over half the plate without flaming. The inoculated plates were incubated at 36°C ± 1°C for 24 hours and the plates were retrieved and read for significant growth (Barbin et al., 1978; Murray and Niles, 1981; Carricajo et al., 1999; Aspevall et al., 2002; Scarparo et al., 2002). In addition, the colony size, the colour changes if any, colony morphology of the organisms growing on both plates were determined and compared to ascertain similarities and difference regarding the growth-supporting abilities of both media. The results were collected and analysed.

4. Results

Of the Thirty-two urine specimens plated on both media, 25 showed significant bacteriuria on the laboratory prepared Desoxycholate medium but only 21 urine specimens showed significant bacteriuria on the commercial (Oxoid) MacConkey agar. Comparison of the composition of commercial MacConkey agar and laboratory prepared Desoxycholate agar is shown in Table 2. Significant bacteriuria was recorded when 0.01ml of fresh urine yielded confluent or near confluent growth on a half plate indicating a number colonies up to or greater than 1000 (≥1000). Non-significant bacteriuria was recorded when the estimated colony count was less than 1000 colonies/0.01ml of fresh urine. This criterion for assessing significance is the acceptable value of the number of organisms in 1ml of fresh urine when acute cystitis or pyelonephritis exists.

Table 2: Comparison of the composition of commercial MacConkey agar and laboratory-prepared Desoxycholate agar (grams/litre)

Media Components	MacConkey Agar Without Salt (Oxoid Ltd) (g)	Laboratory Prepared Desoxycholate Agar (g)
Peptone	20	10
Lactone	10	10
Bile salts	5	-
Agar	12	20
Neutral Red	0.075	0.035
Yeast extract	-	1
Sodium desoxycholate	-	0.025
Ferric citrate	-	0.25

It has been consistently shown by various surveys that clean-catch mid- stream urine when promptly cultured in healthy individuals rarely yield more than 3000 – 50000 Cfu/ml as a result of unavoidable contamination in the process of collection in an area that has dominant normal flora organisms. This number would represent about 30 – 500 Cfu at most in 0.01ml of fresh urine. On the other hand if there was on going multiplication of urinary pathogens in the bladder or kidney case of cystitis or pyelonephritis, it had been shown that a clean-catch mid-stream urine specimen would not yield less than 100000 Cfu/ml. This number would represent about 1000 Cfu/0.01ml of fresh urine.

With regards to colony sizes and fermentation of lactose, the behaviour of the urinary isolates on both media was substantially the same. The results showed that if the commercial MacConkey medium used as a standard reference in this evaluation were employed in the diagnostic laboratory, a substantial number of patients would be diagnosed falsely as having insignificant bacteriuria as a result of inadequate growth of organisms on the commercial MacConkey medium.

The relative poor performance of the commercial MacConkey medium is most likely due to its deterioration in shipping and storage given its old-age. The detail of the comparative behaviour of urinary pathogens on commercial MacConkey medium and on the laboratory prepared Desoxycholate is shown in Table 3.

Table 3: Comparison of growth characteristics of urinary organisms pPlated on commercial MacConkey agar and laboratory prepared Desoxycholate agar

Urine Specimen No.	Estimate of Colony forming Units (Cfu)/0.01ml of fresh Urine		Significant bacteriuria		Lactose Fermentation		Average Colony Size	
	LPDA	CMA	LPDA	CMA	LPDA	CMA	LPDA	CMA
AA001	≥1000	≥1000	+	+	+	+	+++	++
AA002	≥1000	≥1000	+	+	+	+	++++	++++
AA003	≥1000	≥1000	+	+	+	+	++++	++++
AA004	≥1000	≥1000	+	+	+	+	++++	++++
AA005	≥600	≥600	-	-	+	+	++++	++++
AA006	≥1000	≥1000	+	+	+	+	++++	++++
AA007	≥1000	≥1000	+	+	+	+	++++	+++
AA008	≥30	≥30	-	-	+	+	++++	++++
AA009	≥1000	≥1000	+	+	+	+	++	++
AA010	≥1000	≥1000	+	+	+	+	++	+++
AA011	≥1000	≥1000	+	+	+	+	+++	++++
AA012	≥1000	≥1000	+	+	+	+	++++	++++
AA013	≥1000	≥1000	+	+	+	+	+++	+++
AA014	≥1000	≥1000	+	+	+	+	+++	+++
AA015	≥500	≥500	-	-	+	+	+++	+++
AA016	≥1000	≥1000	+	+	+	+	+++	+++
AA017	≥1000	≥1000	+	+	+	+	++++	++++
AA018	≥1000	≥1000	+	+	+	+	++++	++++
AA019	≥1000	≥1000	+	+	+	+	++++	++++
AA020	≥1000	≥1000	+	+	+	+	++++	++++
AA021	≥1000	≥1000	+	+	+	+	++++	++++
AA022	≥500	≥500	-	-	+	+	++++	++++
AA023	≥1000	≥1000	+	+	+	+	++++	++++
AA024	≥1000	≥1000	+	+	+	+	++++	++++
AA025	≥1000	≥1000	+	+	+	+	++++	++++
AA026	≥100	≥100	-	-	+	+	++++	++++
AA027	≥300	≥300	-	-	+	+	++++	++++
AA028	≥800	≥800	-	-	+	+	++++	++++
AA029	≥1000	≥500	+	-	+	+	++++	++++
AA030	≥1000	≥700	+	-	+	+	++++	++++
AA031	≥1000	≥200	+	-	+	+	++++	++++
AA032	≥1000	≥100	+	-	+	+	++++	++++

++++ = Maximum colony size
+++ = Medium colony size
++ = Small colony size
LPDA – Laboratory prepared Desoxycholate agar
CMA – Commercial MacConkey agar
Cfu – Colony forming unit

5. Discussion

MacConkey has proven its value as a useful primary isolation medium, but it also has its problems. MacConkey agar has a short shelf-life and does not keep well in tropical hot and humid environments. Also, the cost of purchase is rather astronomical. In Nigeria today, 90% of hospitals are under-funded especially the laboratory unit, which is one of the most expensive units to run in a hospital. Urine being one of the most frequently submitted specimens has made MacConkey something that is a necessity in the laboratory quite unaffordable. This has prompted the need to examine cheaper media being prepared from basic laboratory ingredients, which performs the same functions and exhibiting the same or better quality as commercial MacConkey agar and in addition better shelf-life in the tropical climate. Laboratory prepared sodium Desoxycholate agar has shown clearly that a medium prepared from basic laboratory ingredients can perform reasonably well and even better as a primary isolation medium when compared with commercial MacConkey agar (CMA).

The poor performance of the commercial Oxoid MacConkey agar compared to the laboratory prepared Desoxycholate agar was unexpected. The available commercial MacConkey agar was old and caked and as it was common with agar media of this type, it had deteriorated in quality and was beginning to be inhibitory. Its growth promoting quality was therefore less than that of the fresh laboratory prepared medium. In fairness to the commercial medium, if a fresh batch was available for the evaluation, it could have proved equal or even superior to the laboratory prepared Desoxycholate agar. Its poor performance due to old age and deterioration under the harsh tropical climate, buttressed the argument for local preparation of a MacConkey substitute at affordable prices in tropical laboratories.

6. Conclusion

The risk of using outdated commercial MacConkey was highlighted in this investigation as quite a number of cases of significant bacteriuria would have been falsely declared as insignificant bacteriuria with serious consequences for patient care, laboratory records and the reputation of the Hospital. Further evaluation of the laboratory prepared Desoxycholate agar using larger number of specimens is desirable to confirm the efficacy demonstrated in this study.

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Faculty of Science
Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Correspondence to:
Kadafa, A. A
Faculty of Environmental Studies
Department of Environmental Planning and Management, University Putra Malaysia
43400 Darul Eshan, Serdang, Selangor, Malaysia
Phone: +6019-2172925; Email: kwaala@yahoo.com

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