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Full Length Research Paper

Comparative studies on five culture media for bacterial isolation

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The research made comparative studies on five media for bacterial isolation. This study aimed at capturing important comparative data in the various types of media for growth efficiency and specific bacteria identification in clinical microbiology. The sources of the samples were urine, nasal swabs and stool. The totality of 15 samples was plated monthly and 120 samples were studied during an eight month period. The mean bacterial load from the cultures grown over the period from each source was calculated and used for comparative growth efficacy. Dominant colonies were characterized and identified based on morphological features and biochemical tests. A 0.1 ml of 10^{-3} of each bacteria isolate was evaluated for growth potential in triplicate on three different special purpose media. The mean bacteria load from the triplicate cultures was calculated. Salmonella-Shigella agar (SSA); a selective medium had the highest number of bacterial colonies of 2.98 x 10^5 CFU/ ml followed by the enrichment medium; blood agar that had 2.96 x 10^5 CFU/ ml and MacConkey agar (MCA) with 2.93 x 10^5 CFU/ ml. Biochemical identification and characterization of four dominant isolates confirmed the presence of *Staphylococcus aureus, Escherichia coli, Salmonella* and *Shigella.* Growth potential of each medium on the bacterial isolates showed that MacConkey agar recorded the highest growth potential of 8.9 x 10^5 CFU/ ml for *E. coli* followed by Blood Agar that gave 8.8 x 10^5 CFU/ml for *Shigella.* The third highest growth potential of 8.6 x 10^5 CFU/ ml was recorded in nutrient agar against *S. aureus.* Statistically, there exists a significant difference among the mean of the five media in their support for bacteria growth at α =0.05.

Key words: Bacterial isolation, isolates, *Escherichia coli*, *Shigella*, Blood agar.

INTRODUCTION

Different types of bacteria that cannot be covered by a single growth medium are found in one sample; therefore, it is important to compare the growth efficacy of routinely used media in clinical microbiology. Some experts in clinical microbiology consider the microbial density to be critical in predicting wound healing and infection while others consider the types of microorganisms to be of greater importance. Infections in clinical microbiology are frequently polymicrobial involving numerous microorganisms that are potentially pathogenic (Bowler, 1998; Bowler and Davies, 1999; Summanen et al., 1995). There has been a debate about the sampling

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technique required to provide the most meaningful data in polymicrobial infection (Bowler et al., 2001). Thus, concern among health care practitioners regarding rapid specific bacteria identification and growth efficiency in clinical microbiology is justifiable. Regarding the role of the microbiology laboratory, consideration must be given to meaningful comparative data in the various types of media for specific bacteria identification in clinical microbiology. Hence, this study aims to capture important comparative data in five types of media for growth efficiency and specific bacteria identification in clinical microbiology.

Dependability on media for isolation of specific bacteria is an important problem for all bacteriology laboratories. Individual enrichment and plating media have been investigated in numerous studies; Orji et al. (2007) reported a significant increase in bacterial isolation when solid media culture was pre-enriched than when the former was used alone. Dunn and Martin (1971) reported that shigellae were best isolated by direct inoculation, whereas salmonellae were isolated in greater numbers after tetrathionate (without Brilliant Green) enrichment with subsequent culturing on the plating medium. Furthermore, Cassar and Cuschieri (2003) studied "Comparison of *Salmonella* Chromogenic Medium (SCM) with desoxycholate citrate lactose sucrose agar (DCLS)". They reported that the sensitivity of SCM was significantly higher after enrichment. In addition, the specificity of SCM was also significantly higher than that of DCLS agar both before and after enrichment. Neil et al. (2014) carried out "Comparison of Blu-ray Disc (BD) MAX Enteric Bacterial Panel (EBP) to Routine Culture Methods for Detection of Campylobacter, Enterohemorrhagic *Escherichia coli* (O157), Salmonella, and Shigella Isolates in Preserved Stool Specimens". The study found that EBP demonstrated superior sensitivity and reliably detected Salmonella, enterohemorrhagic Escherichia coli (EHEC O157), Shigella, and Campylobacter at concentrations 1- to 2-$\log_{10}$ lower than those needed for culture detection. Alo et al. (2013) carried out "a comparative analysis between solid media and liquid media supplementation". The study concluded that the use of broth media to supplement solid media increased the sensitivity of semen culture and higher bacterial isolates were recovered.

Enrichment methods were reported to produce twice the number of pathogens as direct streaking in a study comparing xylose lysine deoxycholate agar, Hektoen enteric agar, Salmonella agar and Eosin methylene blue agar with stool specimes carried out by Taylor and Schelhart (1971)

Various growth media such as blood agar, and MacConkey agar is used for the isolation of gram-negative rods. It also inhibits the growth of gram positive cocci. Blood agar is used to detect the haemolytic streptococci. Instead of using the above mentioned media, some laboratories use single non-inhibitory medium such as cystine lactose electrolyte deficient medium (CLED). Ramzan et al. (2004) carried out comparative study of various growth media in isolation of urinary tract pathogens in which they reported that since different types of organisms are responsible for urinary tract infection, the whole range of pathogens cannot be covered by a single growth medium, therefore, they used blood agar, MacConkey agar and cystine lactose electrolyte deficient medium (CLED). Manipulation of different media and methods for cost-effective characterization of *E. coli* strains collected from different habitats (Arshad et al., 2006) can be done effectively by membrane filtration utilizing three types of selective media and differential agar media (MacConkey, Eosin metylene blue and endo agar) without importing expensive diagnostic kits. The main objective of this study was to provide important comparative information regarding how to choose an appropriate medium for growth of clinical bacterial isolates. This study aims to capture important comparative data in five types of media for growth efficiency vis-a-vis growth potential of each medium in the isolation of *E. coli, S. aureus, Salmonella* and *Shigella.*

MATERIALS AND METHODS

Source of microorganisms

Bacterial populations used in the study were collected from the Anambra State University Medical Centre Laboratory. Samples of urine, nasal swab and stool were collected at random from routine samples submitted for analysis in the Laboratory during the eight month period of the study starting from April, 2011- November, 2011.

Sample processing and Isolation procedure:

Serial dilutions, ratio 1:10 were prepared for each sample. One gram of stool sample was each time suspended in 10 ml of sterile phosphate buffer (pH 7.2) before it was used for serial dilution. Each month, samples were collected from the three sources and inoculated in duplicates on five separate media with 0.1 ml of 10^{-3} dilution. The totality of 15 samples was plated monthly and 120 samples were studied during the period. The media used were all purpose [Nutrient agar (NA)], selective; MacConkey agar (MCA), Mannitol-salt agar (MSA) and Salmonella-Shigella agar (SSA) and Enrichment medium was Blood agar (BA). The plates were incubated for 24 h at 37°C. The mean bacterial load from the cultures made over eight month period from each source was calculated. The media were prepared according to manufacturers' instructions, sterilized and poured onto sterilized Petri dishes.

Evaluation of growth potential of all purpose, selective and enrichment media

Dominant colonies were obtained and used for development of pure cultures which were characterized and identified based on morphological features and biochemical tests (Manaal et al., 2011). Stock cultures of the pure isolates were stored. Subsequently, sub-culturing and reactivation in broth cultures were carried out. Ratios

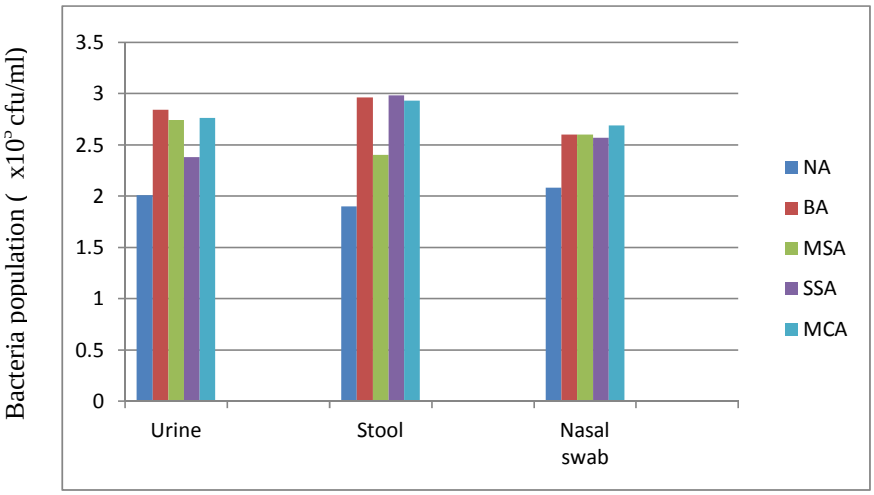

Figure 1. The mean bacterial load from urine, stool and nasal swab samples cultured on different media. NA = Nutrient agar; BA = blood agar; MSA = mannitol salt agar; SSA = Salmonella-Shigella agar; MCA = MacConkey agar.

1:10 dilutions of the reactivated bacteria isolates were made. A 0.1 ml of 10^{-3} of each bacteria isolate was evaluated for growth potential in triplicate on three different special purpose media. The mean bacteria load from the triplicate cultures was calculated.

Statistical analysis

Analysis of variance (ANOVA) for growth efficiency of the types of media in relation with growth potential of each medium in the isolation of *E. coli, S. aureus, Salmonella* and *Shigella* was carried out to deterimine if significance existed among the media in their support for bacteria growth. Tukey test was used to show which media were different. Also standard deviation of bacterial load from urine, stool and nasal swab samples cultured on the five media and standard deviation of growth potentials of the media on four dominant bacterial isolates were calculated.

RESULTS

The mean bacterial load from urine, stool and nasal swab samples cultured on different plating media showed that SSA; a selective medium had the highest number of bacterial colonies of 2.98 x 10^5 CFU/ ml followed by the Enrichment medium (blood agar) that had 2.96 x 10^5 CFU/ ml and MCA with 2.93 x 10^5 CFU/ ml; all were stool samples while Nutrient agar had the least number of bacteria count ranging from 1.90 - 2.08 x 10^5 CFU/ ml (Figure 1). Biochemical identification and characterization of four dominant isolates confirmed the presence of *S. aureus, E. coli, Salmonella* and *Shigella*.

Determination of the growth potential of each medium on the bacterial isolates showed that MacConkey agar recorded the highest growth potential of 8.9 x 10^5 CFU/ ml for *E. coli* followed by BA that gave 8.8 x 10^5 CFU/ ml for *Shigella.* The third highest growth potential of 8.6 x 10^5 CFU/ ml was recorded in NA against *S. aureus* (Figure 2).

Comparative study of the growth potential of the different media is useful in getting information on the microbial density of infection, types of microorganisms and polymicrobial nature of infection.

Statistical analysis using ANOVA showed that there exists a significant difference among the mean of the five media in their support for bacteria growth at α =0.05 and significant value was 0.002. Therefore, null hypothesis was rejected and the rejection of the null hypothesis implies that among the media, there were at least two that had different means. The Turkey test was used for interpretations of multiple comparisons and to show which media were different. Inference showed that the difference in mean performance of each of the following pairs of media is significant; NA and BA, NA and MSA, NA and SSA, NA and MCA. Blood agar, MSA, SSA and MCA with mean 2.800, 2.5667, 2.6433 and 2.7933 respectively performed better than NA with mean 1.9967.

ANOVA result for growth potentials of the different media on four dominant bacterial isolates failed to reject null hypothesis because it had significant value of 0.233. It is not less than significant level, 0.05. Hence, it was concluded that the mean of the five sample media were the same. The standard deviation of the bacterial load from urine, stool and nasal swab samples cultured on the five media was 0.34529 and Standard Deviation of growth potentials of the media on four dominant bacterial isolates was 3.29515.

DISCUSSION

Different types of bacteria are found in urine, stool and nasal cavity, although some of these bacteria are

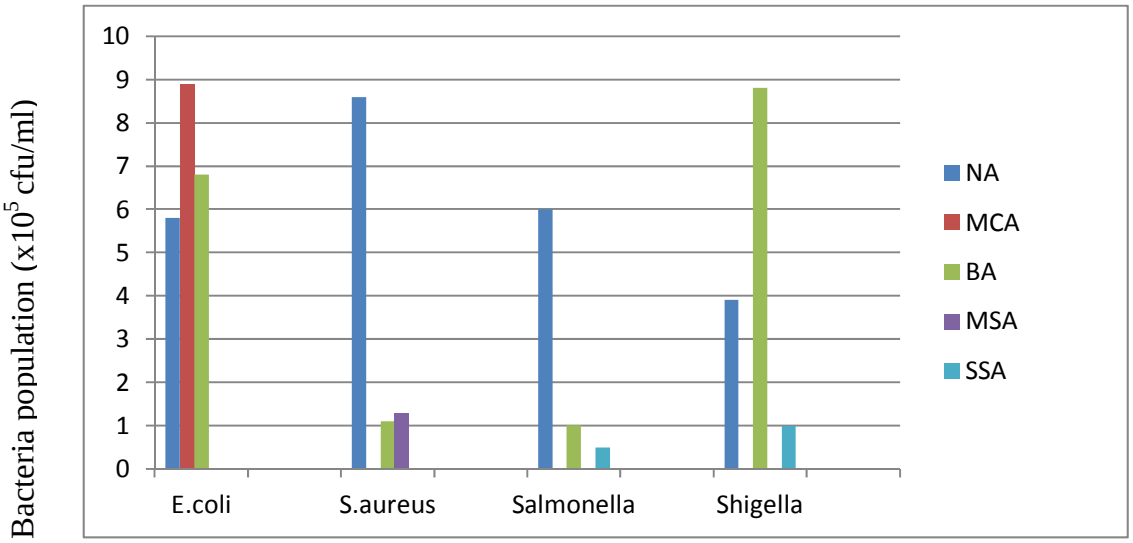

Figure 2. Growth potentials of the different media on four dominant bacterial isolates. NA = Nutrient agar; BA = blood agar; MSA = mannitol salt agar; SSA = Salmonella-Shigella agar; MCA = MacConkey agar.

asymptomatic. Nonetheless, the different types of bacteria found in one sample cannot be covered by a single growth medium; therefore, the observation of this study on mean bacterial load from urine, stool and nasal swab samples cultured on different media lends more weight to the report of Ramzan et al. (2004).

The different sources of the isolates was in line with those of Abdulhadi et al. (2008), who reported that microorganisms colonize different habitats and that the nose is colonized by different microorganisms including *S. aureus*. Similarly, study done by Manaal et al. (2011) reveals *E. coli* as the main causal agent of uniary tract infection and has been isolated from urine. This also agrees with findings of Nicolle (2008). Other studies have revealed the isolation of Salmonella and Shigella from stool specimens. Several different plating media were used for their isolation and Salmonella and Shigella agar was included (Isenberg, 1992; Taylor and Schelhart, 1971; Vandeizant and Splittsoesser, 1992).

The observation that selective and enrichment media are best for isolation of bacteria during routine laboratory investigations lends more weight to previous studies that reported the use of at least one selective media with other plating media during routine laboratory work investigation (Rubina et al., 2006; Olle et al., 2002; Taylor and Schelhart, 1971; Cassar and Cuschieri, 2003).

Similarly, the report of this study that MacConkey agar recorded the highest growth potential for *E. coli* supports the observation of previous researchers (Olle et al., 2002).

Conflict of Interests

The authors have not declared any conflict of interests.

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