

Evaluation of Chicken Head and Foot Extract as a Cost-Effective Alternative to Commercial Microbiological Media for Clinical Isolates

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Abstract

Background: This study investigated the potential of chicken head and foot extract (CHFE) as a low-cost alternative to commercial microbiological media for cultivating clinically important bacteria.

Methods: The growth characteristics, colony morphology, and antibiotic susceptibility profiles of selected pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Salmonella typhi*, and *Shigella dysenteriae*, were assessed on CHFE agar and broth. The growth of *S. aureus*, *E. coli*, and *P. aeruginosa* on CHFE media was comparable to that on Nutrient agar and broth, with characteristic pigment production and colony morphology. However, the growth of *A. baumannii* and *S. dysenteriae* was relatively low on CHFE medium. Supplementation of CHFE broth with 1% glucose or peptone resulted in enhanced growth, surpassing that in Nutrient broth by 20-30%.

Results: Principal-component analysis demonstrated strong relationships between optical density (OD₆₀₀) and colony-forming units (CFU/mL) in both media. Statistical analysis revealed that peptone supplementation had the most pronounced effect on growth improvement. The study highlights the potential of CHFE as a cost-effective medium for clinical diagnostics in resource-limited settings, although further optimization is required to support the growth of fastidious organisms.

Conclusion: The utilization of poultry waste in microbiological media can add value to this waste while reducing the reliance on expensive commercial media in low-income countries.

Keywords: Chicken waste; Microbiological media; Clinical isolates; Poultry waste; Growth characteristics

Introduction

Culture-based diagnostics is still considered as the gold standard for many infectious diseases, such as for Typhoid and Tuberculosis. Culturing is also required to investigate antimicrobial susceptibility of pathogens aiding the physicians to prescribe antibiotics correctly.¹ Traditionally, the diagnostic laboratories use media prepared from complex and refined sources, such as Nutrient agar, Blood agar and MacConkey's agar. These refined sources include peptone, meat extracts, defibrinated blood and other ingredients.¹ With an ever-increasing population and diagnostic facilities, particularly in post-Covid era, the diagnostic labs in low-income countries look for affordable microbiological media.² It is also of particular interest that most of these countries depend on imported media that incurs burden on their meagre foreign exchange reserves.¹ Therefore, development of cost-effective media has remained a research quest in the laboratories around the globe.

In this context, agro-wastes have been described more frequently for their utilization in microbiological media compared to waste from animal sources. However, availability of plant wastes may remain subject to seasonal variations and the composition may differ from crop to crop.³ In contrast, slaughterhouse and poultry wastes are easy to collect and abundantly available in most parts of the world. Poultry waste, in particular, is more readily available, owing to the higher growth rate of the birds compared to grazers and is not affected by the seasonal changes.⁴ Chicken waste is protein rich and contains trace mineral and vitamins, therefore, supports the growth of a variety of microorganisms.⁵ Indeed the growth on chicken waste is comparable to the growth on peptone rich media.⁴ The waste containing media have also been investigated for their suitability to support the growth of clinically important bacteria.⁶ For instance, *E. coli* and *Staphylococcus aureus* were successfully isolated from clinical samples using animal based extracts.⁷ While the use of cow dung extract found useful to obtain enteric pathogens.⁸

Poultry waste, in particular, has been described for its growth promoting effects on *Salmonella*, *Shigella* and *Pseudomonas* species; the authors reported a comparable account with commercially used media.⁹ Not only common pathogens, spent chicken tissue hydrolysate has also been used to grow fastidious organisms including *Haemophilus influenzae* and *Neisseria gonorrhoeae*.⁵ Therefore, waste from chicken waste can be used to formulate bacteriological media which can help diagnostic laboratories in low income countries, including the current study area, Iraq, where limited laboratory resources, financial constraints and certain trade restrictions hinder microbiological diagnosis of infectious diseases and antimicrobial susceptibility testing.¹⁰ This approach can help health care setting to avoid financial burden and frequent stockouts. Therefore, this study was designed to use extracts prepared using chicken head and foot for their growth supporting abilities in microbiological medium. The clinical isolates were cultivated on the chicken waste media and their growth was compared with the commercial media. Considering the large-scale availability of poultry waste in Iraq and other developing, this study holds significance in medical and clinical sciences.

Materials and Methods

Collection of Chicken waste and bacterial isolates

Chicken waste, mainly head and foot were collected locally. After collection, the waste was transported to the laboratory in a sealed plastic bag to avoid further contamination. The bags were kept at 4 °C until used. The processing of this waste was carried out within 24 h of collection.

The bacterial isolates were obtained from the laboratory of a tertiary care hospital in Basrah, Iraq. The isolates were identified through biochemical and microbiological characteristics.

Preparation of Chicken head and foot extract (CHFE)

Chicken head and foot were washed thoroughly with distilled water to remove any visible dirt or other contaminants. To prepare CHFE, ~2 kg of the waste was boiled in 5 L of distilled water for 2 h. This boiling released soluble proteins, amino acids, minerals and other nutrients from the waste. This resultant extract was cooled at room temperature, filtered through four-layers of muslin cloth, centrifuged at 1000 xg for 15 min to obtain a clear preparation. This CHFE was sterilized by filtration through 0.22 μ m filters and stored in amber bottles at 4 °C. The CHFE was used within 5 days of preparation.

Media Formulation

CHFE was directly used as a broth or was added with 1.8% w/v agar technical to solidify it. The extract as amended when required, with 0.5% peptone and 1% w/v glucose. For comparison, commercially available media including Nutrient broth, Nutrient agar and Blood agar were also used.

Retrieval of clinical isolates

The clinical isolates were retrieved from the diagnostic laboratory and were included, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Salmonella typhi* and *Shigella dysenteriae*. The cultures were grown in Nutrient broth for 24 h at 37 °C and the turbidity was adjusted to 0.5 McFarland standard (representing 1.5×10^8 CFU/mL) as given by CLSI guidelines (2023).

Cultivation on CHFE media

To assess the growth of bacterial isolates on CHFE media, 0.1 mL inoculum was streaked on CHFE agar medium following quadratic streaking techniques. The isolates were also streaked on Nutrient agar and Blood agar (for *S. pyogenes* and *S. aureus*). The plates were incubated at 37°C for 24 h and growth was recorded. In addition to

colonial morphology, pigment production, swarming and hemolysis was also monitored. The growth was qualitatively mentioned as heavy growth (+++), moderate growth (++), low growth (+) or no growth (-).

The bacterial growth was also evaluated in CHFE broth for which 0.1 mL inoculum was separately transferred to 10 mL of the CHFE and Nutrient broth. The tubes were incubated at 37 °C for 24 h. After incubation, OD₆₀₀ was noted. For viability assay, a portion (0.1 mL) from this broth was diluted to 10⁻⁸ and streaked on Nutrient agar plates and CFU/mL was counted.

Statistical Analysis

Each experiment was conducted in triplicate and the data has been represented as mean $\pm$ standard deviation. Comparative analysis was evaluated by ANOVA and Tukey's post-hoc test. Data analysis was performed at a 95% confidence interval, and $p < 0.05$ was considered statistically significant

Results

Growth on CHFE agar

CHFE agar was initially tested for its potential to support the growth of clinical isolates. The growth was visually monitored and characteristics of the isolate was observed. The extent of growth of *S. aureus* and *P. aeruginosa* was similar to the growth on Nutrient agar with characteristic golden (Fig. 1) and green pigments respectively (Table 1). It indicated about the provision to use CHFE in clinical settings with rapid identification. While the growth of gram negatives, particularly, *A. baumannii* and *S. dysenteriae* was relatively low on CHFE medium. Despite less growth of *K. pneumoniae* and *P. mirabilis* on CHFE medium, the characteristic mucoid colonies and swarming, respectively was evident. Nonetheless, further optimization is required to demonstrate utility of this medium for the growth of variety of clinical isolates.

Growth in CHFE broth

The bacterial isolates were also cultivated in CHFE broth for 24 h and OD₆₀₀ was noted. An aliquot of 100 μ L was transferred to agar medium and CFU/mL was counted. The results showed that the growth of *S. aureus*, *E. coli* and *P. aeruginosa* was comparable (~90%) to that of the growth on Nutrient broth (Table 2). Whereas, the growth of *S. dysenteriae* reduced to ~55% when compared with the OD₆₀₀ obtained in Nutrient broth. While for other organisms a moderate growth of ~70-80% of the growth obtained on Nutrient broth was observed.

The estimation of CFU/mL of the aliquot from the broth media showed that the count remained higher (in order of 10⁷-10⁸) for all the organisms. However, when it was compared with the CFU/mL obtained on Nutrient agar, it was lower for *P. mirabilis*, *A. baumannii*, *S. typhi* and *S. dysenteriae*. Whereas, other organisms showed CFU/mL similar to obtained on Nutrient agar affirming the suitability of the CHFE media.

Principal-component analysis (PCA) demonstrated strong pair-wise relationships between the OD₆₀₀ and CFU/mL in the same medium (Fig. 2). With regression values >0.9, the data showed that either of the parameter (OD or CFU/mL) is sufficient to express the growth. The PCA showed that *S. aureus* and *P. aeruginosa* are the strong grower on CHFE medium while *A. baumannii* and *S. dysenteriae* remained at low growth extreme.

Effect of supplementation of glucose and peptone in CHFE broth

Consequent to the finding that only OD₆₀₀ can demonstrate the growth of the isolates in the formulated medium or in Nutrient broth, the effect of supplementation of a simple carbon source, glucose, and a complex nitrogen source, peptone, was studied by monitoring OD₆₀₀ in the supplemented medium and by comparing it with the Nutrient broth. The data in the figure 3 present the growth promoting effect of supplemented glucose and peptone alone or in combination. For all the isolates, supplementation of 1% glucose or peptone resulted in the growth higher than the growth in Nutrient broth medium. The growth surpassed 1.2 OD₆₀₀ and was ~20-25% higher than the growth in Nutrient broth for the organisms *S. aureus*, *E. coli* and *P. aeruginosa* in CHFE supplemented with glucose and peptone both. While, the growth approached to 0.9-1.0 OD for *A. baumannii*, *S. typhi* and *S. dysenteriae* in CHFE supplemented with glucose and peptone medium, indicated a ~30% enhancement from the medium without supplementation.

However, statistical analysis of supplementation of glucose and peptone, alone or in combination, elaborated it differently (Fig. 4). The effect of peptone supplementation to CHFE medium was the most pronounced ($F \sim 73.5$ and $p = 3 \times 10^{-6}$) while glucose improved the growth of the isolates modestly ($F \sim 9.1$ and $p = 0.006$). Yet the combined effect of glucose and peptone was not significant ($p = 0.087$), rather it appeared as an additive effect. It shows that the CHFE medium supplemented with peptone only can serve as a growth promoting medium. Nonetheless, growth kinetics data and supplementation of more nutrients need to be investigated to further improve the growth.

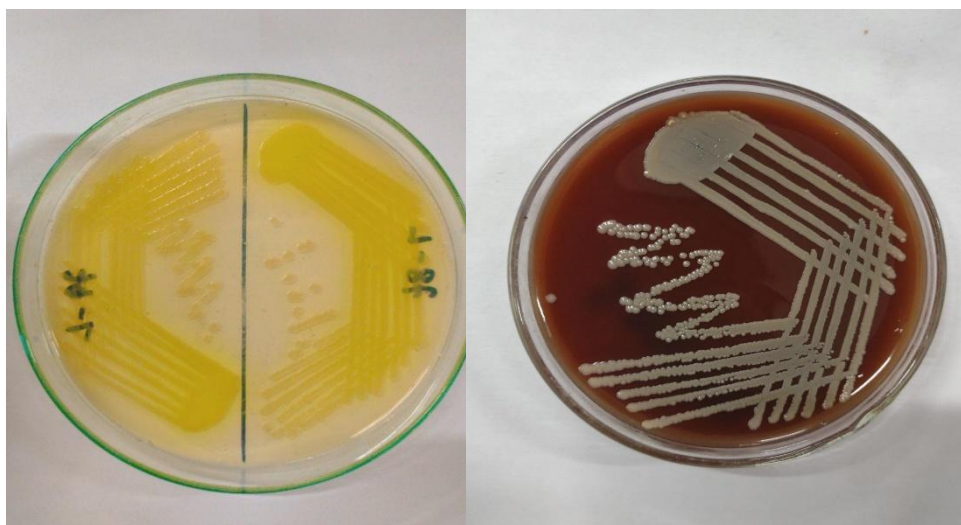
Table 1. Growth of bacterial isolates on Chicken head and foot extract (CHFE) agar. The growth was visually assessed and compared with the growth on Nutrient agar and/or Blood agar.

Bacterial isolate	Growth on CHFE agar*	Distinct feature on CHFE agar	Growth on Nutrient agar	Growth on Blood agar
<i>Staphylococcus aureus</i>	+++	Golden pigment	+++	+++ (b-hemolysis)
<i>Escherichia coli</i>	++	None	+++	ND
<i>Pseudomonas aeruginosa</i>	+++	Green pigment	+++	ND
<i>Klebsiella pneumoniae</i>	++	Mucoid colonies	+++	ND
<i>Proteus mirabilis</i>	++	Reduced swarming	+++ (Extensive swarming)	ND
<i>Acinetobacter baumannii</i>	+	None	++	ND
<i>Salmonella typhi</i>	++	None	++	ND
<i>Shigella dysenteriae</i>	+	None	++	ND

*+++ = extensive growth; ++ = moderate growth; + = scanty growth; ND = not detected (not used for these organisms)

Table 2. Optical density (OD₆₀₀) at 600 nm of the isolates in Chicken head and foot extract broth and Nutrient broth. The viability was assessed by estimating CFU/mL of 0.1 mL aliquot on agar medium.

Organism	OD ₆₀₀ in		CFU/mL in	
	CHFE Broth	Nutrient Broth	CHFE	Nutrient Broth
<i>S. aureus</i>	0.88 ± 0.03	0.93 ± 0.01	1.4×10 ⁸ ± 0.2×10 ⁷	1.5×10 ⁸ ± 0.1×10 ⁷
<i>E. coli</i>	0.75 ± 0.02	0.89 ± 0.02	1.1×10 ⁸ ± 0.3×10 ⁷	1.5×10 ⁸ ± 0.2×10 ⁷
<i>P. aeruginosa</i>	0.92 ± 0.01	0.95 ± 0.02	1.5×10 ⁸ ± 0.1×10 ⁷	1.6×10 ⁸ ± 0.1×10 ⁷
<i>K. pneumoniae</i>	0.68 ± 0.03	0.88 ± 0.01	1.0×10 ⁸ ± 0.2×10 ⁷	1.4×10 ⁸ ± 0.2×10 ⁷
<i>P. mirabilis</i>	0.60 ± 0.04	0.86 ± 0.03	9.2×10 ⁷ ± 0.3×10 ⁷	1.3×10 ⁸ ± 0.1×10 ⁷
<i>A. baumannii</i>	0.42 ± 0.03	0.66 ± 0.02	5.8×10 ⁷ ± 0.2×10 ⁷	9.8×10 ⁷ ± 0.2×10 ⁷
<i>S. typhi</i>	0.55 ± 0.02	0.79 ± 0.01	8.3×10 ⁷ ± 0.1×10 ⁷	1.2×10 ⁸ ± 0.2×10 ⁷
<i>Sh. dysenteriae</i>	0.39 ± 0.03	0.70 ± 0.03	4.9×10 ⁷ ± 0.2×10 ⁷	1.0×10 ⁸ ± 0.1×10 ⁷

**Fig. 1** Growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on CHFE medium expressing pigment.

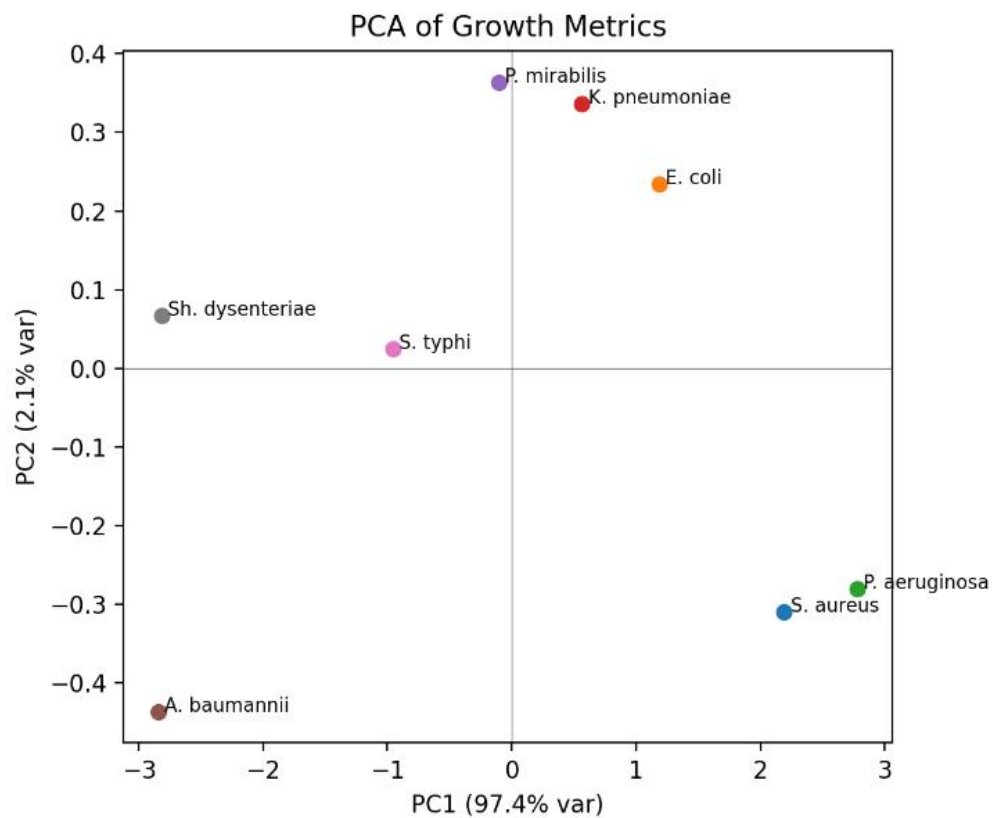


Fig. 2 Principal-component map of growth metrics (OD_{600} and CFU in both media). PC1 (97 % variance) represents overall growth strength—points farther right grew better in both broths—while the much smaller PC2 (2 % variance) captures minor differences between strains.

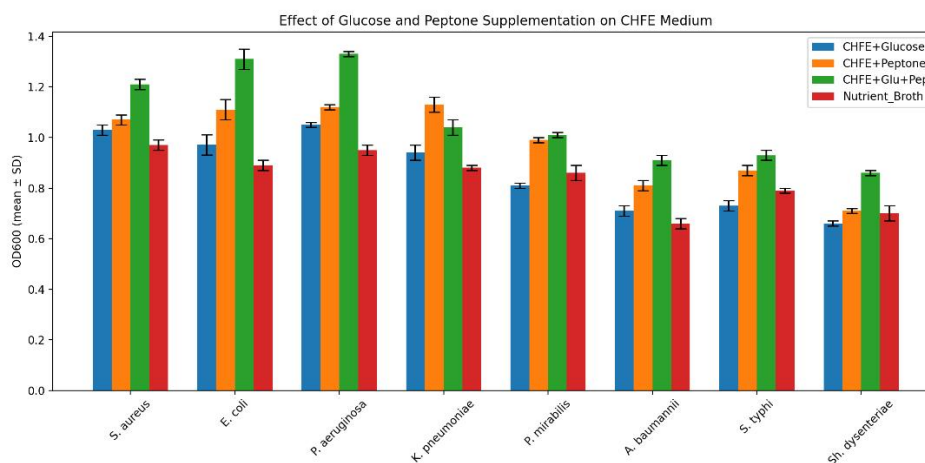


Fig. 3 Growth of clinical isolates in chicken head and foot extract medium with and without supplementation of glucose (Glu) and peptone (pep). The isolates were grown in the medium and turbidity was observed by taking optical density at 600 nm (OD_{600}). The results were compared with the growth in Nutrient broth

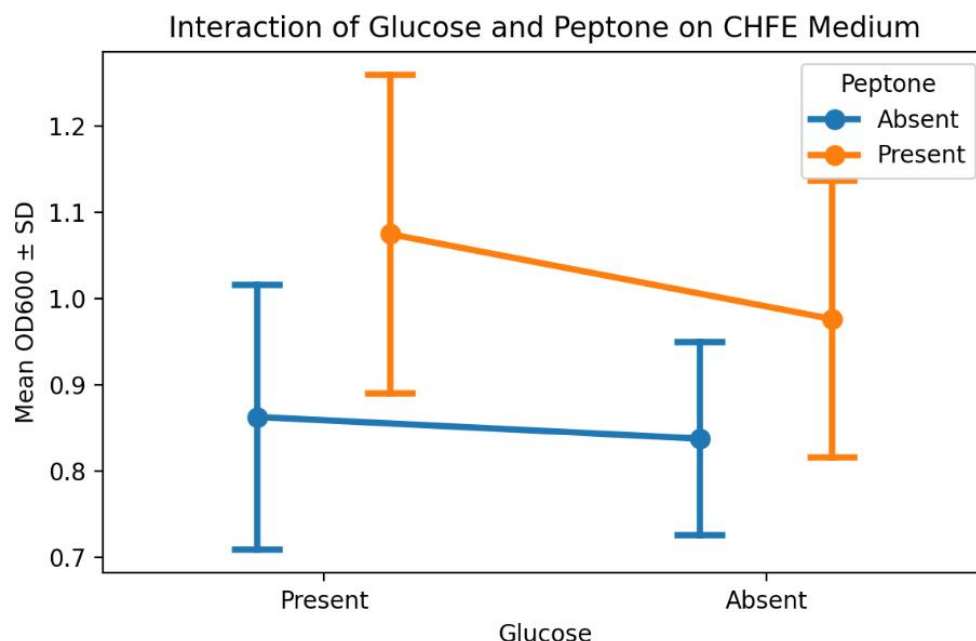


Fig. 4 Individual and combined effect of glucose and peptone supplementation in Chicken head and foot extract medium. Each point represents mean OD of all the organism.

Discussion

This study demonstrated the potential of CHFE as a low-cost medium and an alternative to commercially used bacteriological medium such as Nutrient agar. Poultry waste is indeed considered as difficult to dispose owing to its organic matter and Biological Oxygen Demand (BOD).¹¹ However, its utilization as an alternative to commercial microbiological media can add value to this waste. The high organic matter in chicken waste indicated about the adequate level of nutrition in CHFE medium. Although it is perceived as rich in protein, yet the data of OD₆₀₀ and CFU/mL affirmed its appropriate nutritional contents. Notably, the organisms retained their ability to produce pigment and mucoid colonies on CHFE medium. It showed that the medium can be used effectively to observe phenotypic characteristics of clinically important bacteria.¹³ It also highlighted that CHFE medium can support the production of secondary metabolites affirming a previous finding where non-commercial media supported the pigment production.^{13,14} The mucoid colonies of *K. pneumoniae* confirmed the presence of enough nutrition in CHFE medium for this organism as capsule formation is only observed in presence of sufficient nutritional level.¹⁵ Yet, some organisms did not grow well on CHFE medium, such as, *A. baumannii* and *S. dysenteriae*. It can be attributed to the fastidious nature of these organisms requiring additional nutrients.¹⁶ There may also be presence of some inhibitory substances limiting the growth of these organisms and swarming of *P. mirabilis*. Swarming is a complex phenomenon require complex nutrients such as, amino acids, surface wetness and quorum sensing molecules.¹⁷ Nonetheless, the improvements in the nutritional quality of the CHFE medium can help to replace expensive commercial media, such as, Xylose Lysine Deoxycholate (XLD) medium.

The growth of organism was monitored through OD and CFU/mL. For some organisms, such as, *S. dysenteriae*, the low OD and high CFU/mL did not corroborate. It can be explained by the fact that the organism may remain in metabolically dormant state without exhibiting division but produce more colonies when grown on the nutritionally diversified medium.^{15,18} Nevertheless, PCA affirmed that the results presented as OD or CFU/mL are corroborated and either of the two techniques can be used to monitor the growth in CHFE medium.

Considering the low growth of some organisms, a simple carbon source and a complex nitrogen source was added in the CHFE medium to improve the growth of all the isolates. Glucose being readily available carbon source supports the growth of most of the heterotrophs. Its promoting effect in the extract medium formulation has been demonstrated earlier. The generation time has been found to reduce in glucose containing mineral salt medium than the medium with complex carbon sources.⁹

Amongst complex nitrogen sources, peptone is widely used. It is partially digested protein providing a variety of

nutrients including peptides, amino acids, vitamins and minerals.⁷ Its growth promoting effect was pronounced when was used alone, however, it further improved when was used in combination with glucose. Still, the combined effect of glucose and peptone was not significant showing only additive effect of the two ingredients. Hence, the supplementation of sugars can be avoided considering the cost effectiveness of the medium. Yet, CHFE supplemented media, did not utilize other commercial ingredients. Moreover, a dose-dependent effect of the glucose and peptone needs to be investigated in order to reduce the price of these media and to make them widely available.

Conclusion

Chicken head and foot extract (CHFE) medium, particularly when supplemented with glucose and/or peptone demonstrated promising growth promoting characteristics for clinical isolates. The medium also preserved phenotypic features of the isolates highlighting the provision of CHFE in diagnostic set-ups of low-income settings. Further optimization of dose of nutritional supplement, and studies on growth kinetics are required to utilize CHFE based media on large scale.

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