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EVALUATION OF THREE ENRICHMENT BROTHS AND FIVE PLATING MEDIA FOR *SALMONELLA* DETECTION IN POULTRY

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ABSTRACT

We evaluated the effectiveness of Selenite Cystine (SC), Tetrathionate Brilliant Green (TBG) and Rappaport Vassiliadis (RV) broths for *Salmonella* isolation. We also tested three classic plating media, Salmonella-Shigella Agar (SS), Brilliant Green Agar (BGA), Xylose Lysine Desoxycholate Agar (XLD) and two chromogenic agars, Rambach (RA) and CHROMagar Salmonella (CAS). Among 100 poultry carcasses, 29 were positive for *Salmonella* using all plating media combined. RV broth (69%) and TT broth (58.6%) were more effective than SC broth (24.1%). The chromogenic media gave better results than the classic ones with less false-positive colonies. The most effective isolation medium was CHROMagar, where *Salmonella* was identified in 23 (79.3%) of the 29 positive samples, followed by Rambach (48%). Positivity for *Salmonella* using classic media was 13.8% for BGA, 27.6% for SS and 34.5% for XLD.

Key words: *Salmonella*, poultry, plating media, selective enrichment

INTRODUCTION

Foodborne diseases are an important public health problem. *Salmonella* infects approximately 1.4 million people, resulting in several hundred deaths per year just in the United States. A wide variety of selective media have been developed to isolate this microorganism, but not one is considered perfect. Selective enrichment broths lead to increased salmonella numbers because contain inhibitory compounds that limit non-salmonella microorganisms. At present, enrichment broths containing selenite, and both brilliant green and malachite green are recommended (5). Also currently Rappaport-Vassiliadis broth (RV) medium is recommended for *Salmonella* recovery from low and highly contaminated foods, while tetrathionate broth (TT) incubated at 35ºC is indicated for foods with low microbial load, and at 43ºC for high loads. Traditional plating media are based on lactose fermentation and hydrogen sulfide production. Most are nonspecific and expensive, and the identification is time consuming. Since 1990's, several chromogenic media have been developed to detect *Salmonella*, such as Rambach (22), SM-ID (21), CHROMagar (9), ABC Medium (19), Chromogenic *Salmonella* esterase agar (6), and Rainbow Salmonella Agar (15). They are based on a combination of biochemical characteristics and are highly specific.

The objective of this study was to evaluate the effectiveness of three enrichment broths and five plating agars for *Salmonella* isolation from naturally contaminated poultry carcasses.

MATERIALS AND METHODS

Samples

One hundred refrigerated poultry carcass samples were collected from supermarkets, butcheries, and hospital kitchens over a one year period.

Procedures

Two hundred and twenty five mL of buffered peptone broth (Oxoid) and 25g of poultry carcass were homogenized in a

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Stomacher Lab Blender 400 for one minute and incubated for 18-20h at 37ºC (pre-enrichment). After incubation, 0.1 mL was transferred to 10 mL of Rappaport-Vassiliadis broth (RV) (Oxoid) and 1mL to 10 mL of Tetrathionate Brilliant Green broth (TT) (Difco); both were incubated at 42ºC for 24h. Selenite broth (SC) (10 mL) (Oxoid) was inoculated with 1mL of the pre-enrichment broth and incubated at 37ºC for 24 h. After incubation at 37ºC or 42ºC, a loopfull of each broth was plated onto Rambach agar (Merck), CHROMagar Salmonella, SS Agar (Salmonella-Shigella) (Oxoid), Brilliant Green Agar (BGA) (Oxoid) and Xylose Lysine Desoxycholate Agar (XLD) (Oxoid). After incubation, five typical colonies from each agar plate were submitted to biochemical tests using TSI agar (Difco) and API-20E (Biomérieux). The colonies were also submitted to serological tests using polyvalent somatic and flagellar antisera (Probac do Brasil).

Statistical evaluation

The Cochran test was used to compare the proportions of dependent samples from the enrichment media (11); the Z test was used to compare the results from media with or without chromogenic properties (25). The significance level for all tests was 5%.

RESULTS AND DISCUSSION

Twenty nine samples were positive for *Salmonella*. The pathogen was detected in 7 samples (24.1%) when selenite cystine broth (SC) was used. With tetrathionate (TBG) and Rappaport-Vassiliadis (RV), the isolation rate was higher: 58.6% (17 samples) and 69% (20 samples), respectively. The difference between results in TBG and RV was not significant; however both were considered more effective than SC.

Several authors (3,12,14,20,24) observed that RV is the most effective enrichment broth for *Salmonella*. However, in our study the difference between RV and TT was not significant. Vassiliadis (24) tested 2,000 samples of meat products, pig faeces, and sewage and observed that 17% were positive for *Salmonella* with TT, and 25% with RV. Similar results were observed by Pietzsch (20) who analyzed samples of faeces, pork, and chicken skin and observed 48% and 92% positivity with TT and RV, respectively. Beckers *et al.* (3) tested 590 samples of dehydrated vegetables, chicken, egg, pepper, and ground meat and observed that 63% were positive for *Salmonella* when RV was used and 47% when TT was used. June *et al.* (14) and Hammack *et al.* (12) also found that RV and TT at 42°C were better than SC and TT at 35°C for isolation of *Salmonella* from fresh meat and highly contaminated products.

June *et al.* (14) tested three enrichment procedures, RV at 42°C, TT at 35º and 42ºC and SC at 35°C, to assess their effectiveness in recovering *Salmonella* from artificially contaminated oysters, frog paws, mushrooms, shrimps and non-contaminated poultry. Among 1,125 samples, the positivity for *Salmonella* was 36.3% with RV, 27.5% and 32.7% with TT at 35 and 42°C, respectively, and 29.7% with SC.

Blivet *et al.* (4) also observed that SC was less effective than RV. Testing chicken, egg, and turkey samples, they found *Salmonella* in 97.6% of the positive samples using RV, and only 42.2% using SC. However, they noticed that the SC broth was able to detected low numbers (10 to 50 CFU/mL) of some serotypes of *Salmonella*, such as *S.* Gallinarum, *S.* Pullorum, *S.* Typhi and *S.* Paratyphi.

In this study, the lowest recovery of *Salmonella* using SC broth agrees with results of other studies (13). Bailey *et al.* (2) compared the efficiency of SC and TT for the isolation of *Salmonella* from artificially and naturally contaminated food samples. When the authors analyzed contaminated ground meat and hot dogs, SC was more effective for ground meat while TT was more effective for hot dogs. This can be explained by the microbiota found in these foods. In the 70's, Fagerberg and Avens (8) reported that SC was better for certain serotypes of *Salmonella*, while TT was more efficient for others. However, Cox and Mercuri (7) observed that TT was more toxic when the concentration of microorganisms was low. Therefore, SC can be the most effective broth for certain types of food, where the numbers of *Salmonella* are low.

Arroyo and Arroyo (1) analyzed 264 chicken and sheep samples and found *Salmonella* in 83 (31.4%). They used TT at 37ºC, RV at 42ºC and SC at 37 and 43ºC, and observed that SC gave better results than TT or RV, regardless the incubation temperature.

In our study, the chromogenic media were more efficient and presented fewer false positives than the classic *Salmonella* media. Fig. 1 shows that the most effective plating medium was CHROMagar which detected *Salmonella* in 23 out of 29 positive samples (79.3%), followed by Rambach (48.3%). XLD was the best classic medium (34.5%), followed by SS (27.6%), and BGA (13.8%). These results are similar to those reported by Rhodes and Quesnel (23) and Moringo *et al.* (16).

Our data also showed that CHROMagar (CAS) was better than Rambach (RA); This is in agreement with Narquet and

Table 1. Positivity of poultry carcasses for *Salmonella* using, three enrichment broths and five plating media.

	CAS	RA	XLD	SS	BGA
RV	14	11	7	5	3
TBG	10	8	5	4	2
SC	5	2	3	1	-

CAS: CHROMagar; RA: Rambach Agar; XLD: Xylose Lysine Deoxycholate Agar; SS: Salmonella-Shigella Agar; BGA: Brilliant Green Agar; RV: Rappaport Vassiliadis broth; TBG: Tetrathionate Brilliant Green broth; SC: Selenite Cystine broth.

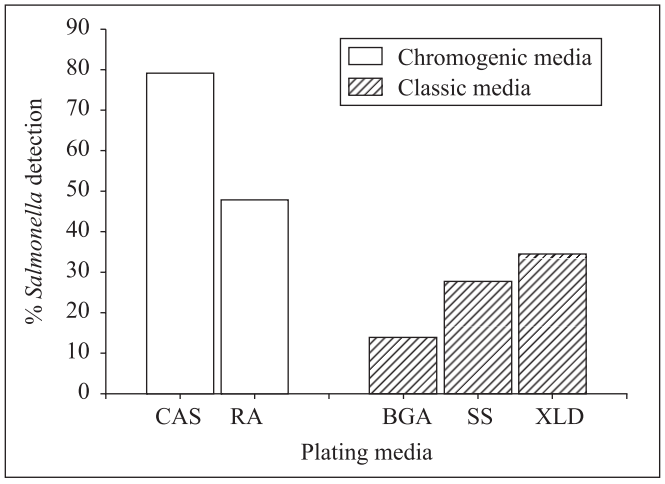

Figure 1. Effectiveness of CHROMagar (CAS), Rambach (RA), Brilliant Green (BGA), Salmonella-Shigella (SS), Xylose Lysine Desoxycholate (XLD) agars for isolation of *Salmonella* from poultry carcasses.

Roupas (17,18), who observed that CAS was able to detect more positive samples than Rambach.

Gaillot *et al.* (9) tested the efficacy of CAS and Hecktoen Enteric Agar (HE) for isolation of *Salmonella* from 508 stool samples. Twenty samples were *Salmonella* positive: CAS detected 19 and HE, 16. The number of false-positive results was lower with CAS than with HE.

In classic media, *Salmonella*, *Proteus* and *Citrobacter* present similar biotypes: they are negative for lactose fermentation and produce H_2S. Since *Proteus* and *Citrobacter* are commonly found in foods, the number of false-positive colonies in classic media may be very large.

False-positive colonies can also occur in chromogenic media: only 21% of the 157 characteristic colonies in RA were confirmed as *Salmonella*. This was due to the large number of *Proteus* and *Citrobacter* in the samples, which can also produce red coloration in this media. According to Rambach (22), these colonies should be blue; however red colonies were also observed by Garrick and Smith (10) and Gaillot *et al.* (9). In this study, *Proteus* colonies presented the same pale-yellow color. The lowest number of false positive colonies was observed in CHROMagar where 82 from 149 were positively identified as *Salmonella*. In this medium, characteristic *Salmonella* colonies had a light violet coloration, while other enterobacteria, including *Proteus* and *Citrobacter*, presented a blue color. False positive colonies that were not *Proteus* or *Citrobacter* were mainly *Pseudomonas aeruginosa* or other small Gram negative rods. Gaillot *et al.* (9) also observed that *Pseudomonas aeruginosa* and *Aeromonas hydrohila* can grow in this medium; the small rods seen in our study may belong to these two species.

Therefore, for *Salmonella* to be detected efficiently, besides two enrichment broths incubated at different temperatures, a chromogenic plating medium should also be used.

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RESUMO

Avaliação de três caldos de enriquecimento e cinco meios de cultura para detecção de *Salmonella* em carcaças de frango

A eficiência dos caldos selenito cistina (SC), tetrationato verde brilhante (TBG) e Rappaport Vassiliadis (RV) foi avaliada quanto ao isolamento de *Salmonella*. Também foram testados três meios clássicos de isolamento, ágar Salmonella-Shigella (SS), ágar Verde Brilhante (VB) e ágar xilose lisina desoxicolato (XLD) e dois meios cromogênicos, Rambach (RA) e CHROMagar (CAS). Entre 100 carcaças de frango examinadas, 29 foram positivas para *Salmonella* usando todos os meios combinados. Os caldos RV (69%) e TT (58,6%) foram mais eficientes que o SC (24,1%). Os meios cromogênicos mostraram melhores resultados do que os clássicos na detecção de *Salmonella* e apresentaram uma quantidade menor de colônias falso-positivas. O meio cromogênico mais eficiente foi o CAS, que detectou *Salmonella* em 23 das 29 amostras positivas (79,3%), seguido pelo RA (48%). Entre os meios clássicos, a detecção foi de 13,8% para VB, 27,6% para SS e 34,5% para XLD.

Palavras-chave: *Salmonella*, aves, meios de isolamento, enriquecimento seletivo

REFERENCES

1. Arroyo, G.; Arroyo, J.A. Efficiency of different enrichment and isolation procedures for the detection of *Salmonella* serotypes in edible offal. *J. Appl. Bacteriol*., 79, 360-367, 1995
2. Bailey, J.S.; Chiu, J.Y.; Cox, N.A.; Johnston, R.A. Improved selective procedure for detection of *Salmonella* from poultry and sausage products. *J. Food Protect.*, 51, 391-396, 1988.
3. Beckers, H.J.; Roberts, D.; Price, O.; Beremer, R.R.; Peter, R. Evaluation of reference material for the detection of *Salmonella*. *Int. J. Food Microbiol*., 3, 287-298, 1986.
4. Blivet, D.; Salvat, G.; Humbert, F.; Colin, P. Evaluation of a new enrichment broth for the isolation of *Salmonella* spp from poultry products. *Int. J. Food Microbiol.*, 38, 211-216, 1997.
5. Busse, M. Media for *Salmonella*. *Inter. J. Food, Microbiol.*, 26, 117-131, 1995.
6. Cooke, V.M.; Miles, R.J.; Price, R.G.; Richardson, A.C. A novel chromogenic ester agar medium for detection of *Salmonella*. *Appl. Environ. Microbiol*., 65, 807-812, 1999.
7. Cox, N.A.; Mercuri, A.J. Recovery of *Salmonella* from broiler carcasses by direct enrichment. *J. Food Protect*., 41, 521-524, 1978.

8. Fagerberg, D.J.; Avens, J.S. Enrichment and plating methodology for *Salmonella* detection in food: A review. *J. Milk Food Technol*., 39, 628-646, 1976.
9. Gaillot, O.; Camillo, D.; Berche, P.; Courcol, R.; Savage, C. Comparison of CHROMagar *Salmonella* Medium and Hektoen Enteric Agar for isolation of Salmonella from stool sample. *J. Clin. Microbiol*., 37, 762-765, 1999.
10. Garrick, R.G.; Smith, A. Evaluation of Rambach agar for the differentiation of *Salmonella* species from other Enterobacteriaceae. *Lett. Appl. Microbiol*., 18, 187-189, 1994.
11. Goodman, LA. Simultaneous confidence intervals for contrasts among multinomial populations. *Ann. Math. Stat.*, 35(2), 716-25, 1964.
12. Hammack, T.S.; Amaguana, R.M.; June, G.A.; Sherrod, P.S.; Andrews, W.H. Relative effectiveness of selenite cystine broth, tetrathionate broth and Rappaport-Vassiliadis medium for recovery of *Salmonella* spp from foods with a low microbial load. *J. Food Protect*., 62, 16-21, 1999.
13. Humbert, F.; Morvan, H.; Lalande, I.; Colin, P. 1995 apud Blivet, D.; Salvat, G.; Humbert, F.; Colin, P. Evaluation of a new enrichment broth for the isolation of *Salmonella* spp from poultry products. *Int. J. Food Microbiol*., 38, 211-216, 1997.
14. June, G.A.; Sherrod, P.S.; Hammack, T.S.; Amaguana, R.M.; Andrews, W.H. Relative effectiveness of selenite cystine broth, tetrathionate broth and Rappaport-Vassiliadis medium for recovery of *Salmonella* spp from raw flesh highly contaminated food and poultry feed: collaborative study. *J. AOAC Intern*., 1307-23, 1995.
15. Manafi, M. New developments in chromogenic and fluorogenic culture media. *Int. J. Food Microbiol*., 60, 205-218, 2000.
16. Moringo, M.A.; Martinez, M.E.; Munoz, A.; Cormax, R. Evaluation of different plating media used in the isolation of salmonellas from environmental samples. *J. Food Protect*., 66, 353-360, 1989.
17. Narquet, F.; Roupas, A. Comparison between Semi-Solid Rappaport-Vassiliadis (SSRV), Rambach agar e CHROMagar for isolation of *Salmonella* from chicken. Public Health Department, Genebra, 1996a.
18. Narquet, F.; Roupas, A. Comparison between Semi-Solid Rappaport-Vassiliadis (SSRV), Rambach agar e CHROMagar for *Salmonella* spp detection. Public Health Department, Genebra, 1996b.
19. Perry, J.D.; Ford, M.; Taylor, J.; Jones, A.L.; Freeman, R.; Gould, F.K. ABC Medium, a new chromogenic agar for selective isolation of *Salmonella* spp. *J. Clin. Microbiol.*, 37, 766-768, 1999.
20. Pietzsch, O, apud BURSE, M. Media for *Salmonella*. *Inter. J. Food, Microbiol*., 26, 117-131, 1984.
21. Poupart, M.C.; Mounier, M.; Denis, F.; Sirot, J.; Couturier; C.; Villeval, F. A new chromogenic ready-to-use medium for Salmonella detection. In Abstracts of the 5Th European congress of clinical Microbiology and Infectious Diseases, Oslo, Norway, 1991.
22. Rambach, A. New plate medium for facilities differentiation of *Salmonella* spp from *Proteus* spp and other enteric bacteria. *Appl. Environ. Microbiol*., 56, 301-303, 1990.
23. Rhodes, P.; Quesnel, L.B. Comparison of Muller-Kauffmann Tetrathionate broth with Rappaport-Vassiliadis (RV) medium for the isolation of salmonellas from sewage sludge. *J. Appl. Bacteriol*., 60, 161-167, 1986.
24. Vassiliadis, P. The Rappaport-Vassiliadis (RV) enrichment medium for the isolation of salmonellas: an overview. *J. Appl. Bacteriol*., 54, 69-76, 1983.
25. Zar, JH. Biostatistical analysis. Prentice Hall, New Jersey, 718p, 1996.