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Assessment of Baird-Parker Agar as Screening Test for Determination of *Staphylococcus aureus* in Poultry Meat

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Baird-Parker agar with egg yolk/tellurite emulsion (BPA) is widely accepted as a medium for the enumeration of *Staphylococcus aureus* in foods. However, it is not completely selective and colonies of other genera or species could be similar to those of *Staphylococcus aureus*. Moreover, the strains of *Staphylococcus aureus* that are lecithinase negative could go unnoticed. Both facts could affect the counts. The aim of this study was to determine whether the enumeration of the colonies with the typical morphology of *Staphylococcus aureus* on BPA is sufficient to quantify this species in poultry meat. Forty chicken carcasses were tested for *Staphylococcus aureus* by surface plating using BPA. Results indicate that the predictive value of the morphology of the colonies on BPA is 85.71% and 68.42% for typical and atypical colonies of *Staphylococcus aureus*, respectively. However, *Staphylococcus aureus* counts (after identification) and counts of typical colonies did not show any significant differences ($P>0.05$) and are significantly ($P<0.001$) correlated ($r = 0.996$). These results suggest that, for screening purposes, enumeration of *Staphylococcus aureus* from poultry meat does not require any identification of strains, resulting in a saving of time and money.

Key words: *Staphylococcus aureus*, poultry, Baird-Parker, screening test

Staphylococcus aureus food poisoning is caused by ingestion of preformed toxin produced in foods (20, 37), and is one of the major causes of foodborne illness throughout the world (18, 23, 26). As a consequence, there is a widespread requirement for reliable methods to detect *Staphylococcus aureus* either for food quality assurance programs or for epidemiological purposes. Their presence in poultry meat (36) emphasizes the need for laboratory surveillance for this bacterial pathogen.

Several selective media for *Staphylococcus aureus* have been developed, but Baird-Parker agar with egg yolk/tellurite emulsion (BPA) (8) remains one of the most frequently used media to detect and enumerate these microorganisms in foods (7). The International Organization for Standardization (ISO) (5), the Association of Official Analytical Chemists International (AOAC) (2), the Bacteriological Analytical Manual (BAM) (10), the American Public Health Association (APHA) (25) and the International Dairy Federation (IDF) (3) all recommend BPA for enumeration and isolation of *Staphylococcus aureus* from foods.

On BPA, *Staphylococcus aureus* reduces tellurite to form grey-black shiny colonies. Colonies are surrounded by an opaque halo (lecithinase activity) and generally show an iridescent film in and immediately around the colonies (lipase activity). BPA is often not sufficiently selective for the analysis of foodstuffs presenting a high level of contaminant flora (38), such as poultry meat. Some of these microorganisms (staphylococci other than *Staphylococcus aureus*, *Bacillus* spp., *Proteus* spp., enterococci or micrococci) show colonies with a similar morphology to those of *Staphylococcus aureus* (4, 14, 21, 22, 31). Moreover, there are *Staphylococcus aureus* lecithinase negative strains (frequent in meat) which do not produce clear zones around the colonies (28). These facts could affect the *Staphylococcus aureus* counts carried out on BPA.

The aim of this work was to assess the validity of Baird-Parker medium as a screening test for the determination of *Staphylococcus aureus* in raw poultry meat. For this, counts based on the morphology of the colonies and counts obtained after identification of strains were compared.

Materials and Methods

Forty eviscerated and refrigerated chicken carcasses were

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purchased from local retailers for microbiological analysis. Samples remained refrigerated during transport and upon arrival at the laboratory. Samples were processed within 4 h. Twenty-five gram portions of breast skin were removed using sterile instruments, and homogenized in a stomacher (Stomacher 400, A.J. Seward, London, England) for 2 min in peptone water (1 : 10 wt/vol, Oxoid Ltd., Hampshire, England). Dilutions were prepared by the transfer of 1 ml of homogenate to 9 ml of peptone water. A volume of 0.1 ml of each dilution was surface plated on Baird-Parker agar containing egg-yolk tellurite emulsion (Oxoid Ltd., Hampshire, England), and incubated at 37°C for 24 and 48 h (25). Duplicate plates were inoculated from each dilution.

For each carcass, a dilution (plates containing 20-200 colonies) was selected. The total number of colonies, colonies with a typical morphology of *Staphylococcus aureus* (T) and colonies with different morphology to those of *Staphylococcus aureus* (atypical, A) were counted. Four colonies (2T and 2A) from each carcass were randomly selected, purified and tested for cell morphology, arrangement of the cells, Gram reaction (19), catalase activity (12), modified oxidase test (16), ability to produce acid anaerobically in a glucose-containing growth medium (15), sensitivity to lysostaphin (34), coagulase activity (11, 24, 25, 27), thermo-stable nuclease activity (30), acid production from maltose and mannitol, and acetoin production (9).

The Gram negative rods were identified using API 20E (bioMérieux S. A., Marcy-L'Étoile, France). The Gram positive bacilli were not identified. The Gram positive cocci were identified using the identification schemes proposed by Evans and Kloos (15), Schleifer and Kloos (34), and Schleifer (33).

After the identification, the percentages of *Staphylococcus aureus*, *Staphylococcus* spp. and *Micrococcaceae* strains in both groups of colonies (T and A) were calculated. These percentages were later used to correct the results of the counts obtained from each BPA plate in the following way :

$$SA = (NT \times \%SA_T + NA \times \%SA_A) / 100$$
$$S = (NT \times \%S_T + NA \times \%S_A) / 100$$
$$M = (NT \times \%M_T + NA \times \%M_A) / 100$$

where :

SA, S, M = number of colonies of *Staphylococcus aureus* (SA), *Staphylococcus* spp (S) or *Micrococcaceae* (M).

NT, NA = number of typical (NT), atypical (NA) colonies

% $(SA, S, M)_T$ = percentage of SA, S or M in typical colonies studied

% $(SA, S, M)_A$ = percentage of SA, S or M in atypical colonies studied

The Student's t-test was computed to examine differences in *Staphylococcus aureus* counts and typical colonies (T) counts. The degree of correlation between both groups was established by linear regression continuous data. The computations were carried out using the Statistica® 6.0 software package (Statsoft Ltd., Chicago, Il, USA).

Results and Discussion

Table 1 shows the identification results of 160 strains isolated from BPA. The predictive value of a typical colony or a positive test (the probability of a typical colony being *Staphylococcus aureus*) is 85.71% and the predictive value of an atypical colony or a negative test (the probability of an atypical colony not being *Staphylococcus aureus*) is 68.42% (Fig. 1 and Table 2).

Eight (9.52%) typical colonies were identified as Gram positive bacilli, and four (4.76%) as staphylococci other

Table 1. Identification of isolates from Baird-Parker agar

Identification	Number (percentage) of strains in colonies :	
	T[1)]	A[2)]
Staphylococcus aureus	72 (85.71)	24 (31.58)
Staphylococci other than *Staphylococcus aureus*	4 (4.76)	32 (42.10)
Staphylococcus spp.	76 (90.48)	56 (73.68)
Micrococcus spp.	0	10 (13.16)
Micrococcaceae	76 (90.48)	66 (86.84)
Proteus spp.	0	4 (5.26)
Gram positive bacilli (non-identified)	8 (9.52)	6 (7.89)
Total	84 (100)	76 (100)

T[1)] grey-black shiny convex colonies surrounded by clear halo; A[2)], colonies with different morphology to T

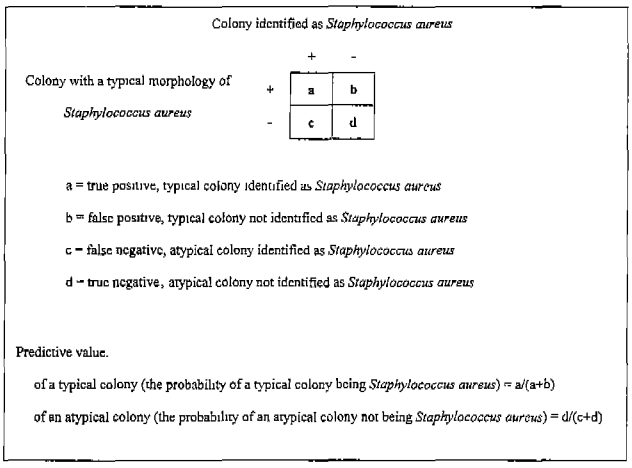


Fig. 1. Definition and calculation of predictive value of a typical and of an atypical colony of *Staphylococcus aureus* on Baird-Parker medium.

Table 2. Evaluation of the differential character of Baird-Parker agar

Isolated colonies	Colonies T[1)]	Confirmed *Staphylococcus aureus*		Predictive value	
		+	-	test +	test -
160	+	72 (a)	12 (b)	85.71%	68.42%
	-	24 (c)	52 (d)		

T[1)], colonies with a typical morphology of *Staphylococcus aureus*: grey-black shiny convex colonies surrounded by clear halo.
For interpretation, see Fig. 1.

than *Staphylococcus aureus*. These results agree with the data in the Oxoid manual (4) where it is shown that *Bacillus* spp. and various species of the genus *Staphylococcus* can form colonies with a similar morphology of lecithinase positive *Staphylococcus aureus* strains on BPA.

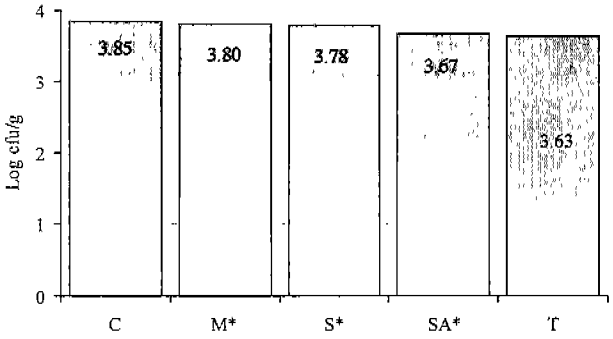
The percentage of *Staphylococcus aureus* in colonies T (85.71%) is greater than those reported by Adesiyun *et al.* (1), who found that between 53% and 57% of the *Staphylococcus aureus* strains isolated from milk samples produced a clear halo on BPA. However, according to these authors, *Staphylococcus aureus* of bovine origin are frequently atypical.

With regard to the atypical colonies, almost all of them, with the exception of *Micrococcus* spp., were black and without a clear halo and indistinguishable from one another. The micrococci produced very small brown colonies. The identification results of the atypical colonies are very similar to those obtained by other authors (4, 17, 21, 22, 28, 31), who show that strains of *Staphylococcus aureus* lecithinase negative, staphylococci other than *Staphylococcus aureus*, *Bacillus* spp., *Proteus* spp., enterococci and micrococci form on BPA black colonies without a clear halo.

The majority of strains isolated (90.48% of colonies T and 73.68% of colonies A) were identified as *Staphylococcus* spp. These results suggest that BPA is a selective medium for staphylococci as it has been previously indicated by other authors (13, 29). The fact that strains of other bacterial genera apart from *Staphylococcus* spp. have grown is not in any way strange because the activity of tellurite can, up to a point, be annulled by the egg yolk (32). Therefore, the selectivity of BPA is often criticized (21, 35). In fact, other culture media, such as rabbit plasma fibrinogen agar medium, have been developed so as to minimize this problem (6).

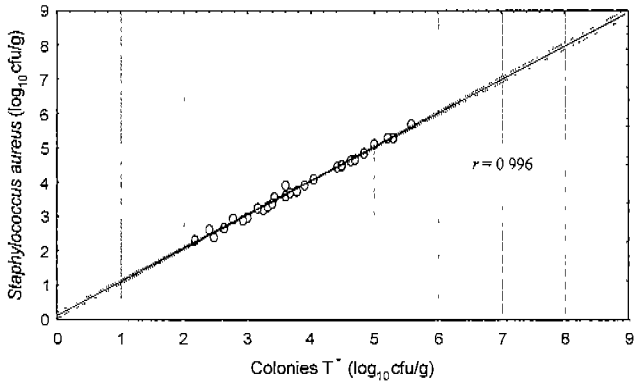
All the typical colonies identified as *Staphylococcus* spp. were coagulase positive. In the atypical colonies, all *Staphylococcus aureus* strains and two staphylococci strains other than *Staphylococcus aureus* were coagulase positive.

Fig. 2 shows average counts ($\log_{10}$ cfu/g) of microbial groups studied. Fig. 3 shows the linear relationship between the *Staphylococcus aureus* counts and the T colonies. As can be seen, the counts of the different microbial groups



C, total colonies on BPA; M*, *Micrococcaceae*; S*, *Staphylococcus* spp.; SA*, *Staphylococcus aureus*; T, typical colonies of *Staphylococcus aureus*. M*, S* and SA*, counts after identification.

Fig. 2. Bacterial counts of chicken breast skin.



T*, colonies with a typical morphology of *Staphylococcus aureus*

Fig. 3. Comparison of typical and confirmed colonies of *Staphylococcus aureus* on chicken breast skin.

studied are very similar. There were no significant differences ($P>0.05$) between the *Staphylococcus aureus* counts (3.67 $\log_{10}$ cfu/g) and the T colonies counts (3.63 $\log_{10}$ cfu/g). Isigidi *et al.* (21) also found minimum differences between both counts. For this reason, and taking into account the high correlation ($r=0.996$) between counts for both groups, we believe that, for screening purposes, to quantify *Staphylococcus aureus* in poultry meat it is sufficient to count the typical colonies on BPA as the identification does not noticeably modify counts and it is an important saving of time and money.

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