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Combined Use of Pastorex Staph-Plus and Either of Two New Chromogenic Agars, MRSA ID and CHROMagar MRSA, for Detection of Methicillin-Resistant *Staphylococcus aureus*[▽]

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We describe the search toward a fast and reliable strategy to detect and confirm the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in screening samples. First, we evaluated the sensitivities and specificities of oxacillin resistance screening agar (ORSA) with enrichment (tryptic soy broth [TSB] and ORSA [TSB-ORSA]) and without enrichment (ORSA), MRSA ID (MRSA_ID) plates, and CHROMagar MRSA (C_MRSA) plates, all of which were inoculated with equal volumes of a suspension made by emulsifying screening swabs. Whereas the sensitivities after 48 h were similar for all media tested (77% for MRSA_ID and ORSA; 73% for C_MRSA and ORSA after enrichment [TSB-ORSA]), the specificities of MRSA_ID (98% after 24 h and 94% after 48 h) and C_MRSA (98% after 24 h and 90% after 48 h) were superior to the specificities of ORSAs (92% after 24 h and 83% after 48 h) and TSB-ORSA (86% after 24 h and 81% after 48 h). Subsequently, the performance of the Pastorex Staph-Plus agglutination test with presumptive MRSA isolates taken directly from chromogenic agars (direct_Pastorex agglutination) was compared to that of the Pastorex Staph-Plus agglutination test with isolates from blood agar subcultures (conventional_Pastorex agglutination). When the direct_Pastorex agglutination test on MRSA_ID plates was combined with Gram staining, the direct_Pastorex agglutination test with samples from MRSA_ID plates was as reliable as the conventional_Pastorex agglutination test with samples from blood agar subcultures from MRSA_ID plates. In contrast, the direct_Pastorex agglutination test with samples from C_MRSA plates gave false-negative results. Finally, we calculated the processing times of the four different strategies, namely, (i) enrichment in TSB supplemented with NaCl, subsequent culture on ORSA, and the conventional_Pastorex agglutination test; (ii) direct inoculation of ORSA combined with conventional_Pastorex agglutination test; (iii) direct inoculation of MRSA_ID plates combined with Gram staining and the direct_Pastorex agglutination test; and (iv) direct inoculation of C_MRSA plates combined with Gram staining and the direct_Pastorex agglutination test. We concluded that the use of MRSA_ID in combination with Gram staining and the direct_Pastorex agglutination test is faster and more specific than the other strategies tested.

In the early 1960s, methicillin resistance appeared among nosocomial isolates of *Staphylococcus aureus* (4). Since then, methicillin-resistant *Staphylococcus aureus* (MRSA) has evolved as one of the most important pathogens in hospitals and intensive care units worldwide. More recently, new strains of MRSA associated with aggressive infections in young, otherwise healthy patients have emerged in the community (7). In order to limit the spread of MRSA, rapid and sensitive tests for the detection of MRSA infection or carriage in both patients and health care workers are required.

The use of mannitol salt agar supplemented with oxacillin and the use of oxacillin resistance screening agar (ORSA) are two of the culture methods most widely used today. However, the use of such mannitol-containing culture agars is complicated by the existence of mannitol-negative *Staphylococcus aureus* strains and mannitol-positive methicillin-resistant coagulase-negative staphylococci (CoNS). Limited sensitivities and specificities have been reported for both culture methods (1, 2).

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In this study we aimed to find a fast and reliable strategy for the detection and confirmation of the presence of MRSA in patients. To achieve this goal we initially evaluated the sensitivity and specificity of ORSA (with and without enrichment), MRSA ID (MRSA_ID), and CHROMagar MRSA (C_MRSA) plates. Subsequently, in order to find a fast and easy confirmatory test, we compared the performance of Pastorex Staph-Plus agglutination tests performed with presumptive MRSA isolates (direct_Pastorex agglutination tests) taken directly from the novel chromogenic agars with that of Pastorex Staph-Plus agglutination tests performed with colonies taken from blood agar subcultures (conventional_Pastorex agglutination tests). Finally, we calculated the processing times of four strategies, namely, (i) enrichment of sample material in tryptic soy broth (TSB) supplemented with NaCl, subsequent culture on ORSA, and Pastorex Staph-Plus agglutination tests performed with colonies taken from blood agar subcultures; (ii) direct inoculation of ORSA plates with sample material and the Pastorex Staph-Plus agglutination test with colonies taken from blood agar subcultures; (iii) direct inoculation of MRSA_ID plates, Gram staining, and Pastorex Staph-Plus agglutination tests with presumptive MRSA isolates taken directly from MRSA_ID plates; and (iv) direct inoculation of C_MRSA plates, Gram staining, and Pastorex Staph-Plus agglutination tests with pre-

TABLE 1. Comparison of results obtained by use of ORSA, MRSA_ID, C_MRSA, and TSB-ORSA[a]

Medium	No. of samples with the following results:						Sensitivity (%)		Specificity (%)	
	True positive		False negative	False positive		True negative				
	22–24 h	48 h		22–24 h	48 h		22–24 h	48 h	22–24 h	48 h
ORSA	17	23	7	25	58	278	57	77	92	83
MRSA_ID	18	23	7	6	20	316	60	77	98	94
C_MRSA	20	22	8	6	34	302	67	73	98	90
TSB-ORSA	21	22	8	46	64	272	70	73	86	81

[a] Outcome of the comparison of ORSA, MRSA_ID, C_MRSA, and TSB-ORSA in terms of true positives, false negatives, false positives, and true negatives. A total of 366 swabs screened for MRSA were used for this comparison. Thirty swabs yielded strains of MRSA confirmed to be MRSA by *mecA* and *nuc* PCRs on one or more media within 48 h of incubation.

sumptive MRSA isolates taken directly from the C_MRSA plates.

MATERIALS AND METHODS

Culture media. Commercial ORSA plates were obtained from Oxoid Ltd. (Basingstoke, England), C_MRSA plates were obtained from Becton Dickinson (Sparks, MD), and MRSA_ID plates were obtained from BioMérieux, Inc. (Marcy l'Etoile, France). MRSA isolates form blue colonies on ORSA and green colonies on MRSA_ID due to mannitol fermentation and the production of alpha-glucosidase, respectively. The ingredients of C_MRSA that cause MRSA strains to turn pink are not disclosed. Both MRSA_ID and C_MRSA contain cefoxitin, whereas ORSA contains oxacillin. TSB (Becton Dickinson) was made in-house, according to the manufacturer's protocol. In order to prepare TSB-NaCl, NaCl was added to TSB to obtain a total NaCl concentration of 7.5%. Blood agars (triple sugar iron plus 5% sheep blood) were obtained from Becton Dickinson.

Study design. (i) Screening samples. A total of 366 swab specimens referred to our laboratory for screening for MRSA were used in this study. These swab specimens were collected in June and July 2005. All specimens were submitted to our laboratory by a pneumatic transport system and were processed within 4 h after collection. Samples were obtained from the following sites: perineum (n = 153), throat (n = 116), nose (n = 54), mouth (n = 30), wounds (n = 8), umbilical region (n = 3), and ear (n = 2).

(ii) Inoculation and incubation of agars. Each swab was emulsified in 600 μl of sterile physiological saline. By using calibrated loops, 10 μl of the resulting suspension was inoculated onto ORSA, C_MRSA, and MRSA_ID plates. In addition, 10 μl of this initial suspension was used to inoculate TSB-NaCl, which was used as an enrichment medium. After 18 to 20 h incubation at 37°C, inoculation of an ORSA plate with this TSB-NaCl medium (referred to as a TSB-ORSA plate) was performed by using a swab. All ORSA, C_MRSA, MRSA_ID, and TSB-ORSA plates were incubated in air at 37°C; the C_MRSA and MRSA_ID plates were kept in the dark for the entire incubation.

(iii) Interpretation of growth on agars and blood agar subcultures. Interpretation of the growth on the agars and blood agar subcultures was done by one laboratory staff member after 22 to 24 h. Blue colonies on ORSA plates, green colonies on MRSA_ID plates, and pink colonies on C_MRSA plates were considered, according to the manufacturers' guidelines, presumptive MRSA isolates and were subcultured on blood agar. In the absence of blue colonies (ORSA and TSB-ORSA plates), green colonies (MRSA_ID plates), or pink colonies (C_MRSA plates), the culture agars were reincubated and the results were reinterpreted after a total incubation time of 48 h. Again, blood agar subcultures were made from all presumptive MRSA isolates. Uncolored colonies on ORSA and MRSA_ID plates and colonies other than pink colonies on C_MRSA plates were not further investigated.

Identification and confirmation strategy. (i) Tests used for confirmation. (a) Tube coagulase test. The tube coagulase test for the detection of free coagulase was performed with rabbit plasma (BioMérieux). One colony was suspended in 0.5 ml of TSB and mixed with 0.5 ml of rabbit plasma, and the mixture was incubated at 37°C. The tubes were inspected for clot formation after 4 and 24 h.

(b) Pastorex Staph-Plus latex agglutination test. The Pastorex Staph-Plus latex agglutination test (Bio-Rad, Marnes-la-Coquette, France) is a rapid latex agglutination test that is based on the detection of clumping factor, staphylococcal protein A, and capsular polysaccharides. The latex agglutination reagent was mixed with colonies taken from blood agar subcultures. This test is referred to here as the conventional_Pastorex agglutination test.

(c) *mecA*/*nuc* PCR. The following primers were used: for *mecA*, primer Mec A1 (AAA ATC GAT GGT AAA GGT TGG C) and primer MecA2 (AGT TCT GCA GTA CCG GAT TTG C), and for *nuc*, primer Nuc1 (GCG ATT GAT GGT GAT ACG GTT) and primer Nuc2 (AGC CAA GCC TTG ACG AAC TAA AGC).

(ii) Identification of MRSA and confirmation of identification. Gram staining and the conventional_Pastorex agglutination test were performed with samples from all blood agar subcultures. A negative conventional_Pastorex agglutination test result was confirmed by the tube coagulase test, and a positive conventional_Pastorex agglutination test result was further investigated by susceptibility testing and the result was confirmed by *mecA* and *nuc* PCRs, unless Gram staining pointed out that the colony examined was not a gram-positive coccus.

(iii) Identification of FP results on MRSA_ID and C_MRSA plates. False-positive (FP) results were identified by combining the Gram staining, catalase reaction, conventional_Pastorex agglutination test, and tube coagulase test results. Conventional tests and API galleries (BioMérieux) were used for the identification of gram-negative rods.

Evaluation of "direct" Pastorex agglutination test. Whenever a sufficient number of colonies was present on the MRSA_ID or C_MRSA plates, presumptive MRSA isolates were taken directly from the chromogenic agar plates and mixed with the Pastorex Staph-Plus latex reagent. This test is referred to as the direct_Pastorex agglutination test. A positive reaction was evidenced by the formation of aggregates within 30 s of the beginning of card rotation and with the reagent test only. The direct_Pastorex agglutination test with samples from MRSA_ID and C_MRSA plates was evaluated by comparing the results with the outcomes of the conventional_Pastorex agglutination test performed with samples from blood agar subcultures on MRSA_ID and C_MRSA plates, respectively.

Calculation of processing time. To calculate the processing time, we examined the time point at which the first result of any confirmatory test that confirmed the presence of an MRSA strain was known. For each strategy, the number of strains detected as well as the cumulative percentage was calculated at four different time points (24 h, 48 h, 72 h, and >96 h).

RESULTS

Evaluation of screening samples with ORSA, C_MRSA, MRSA_ID, and TSB-ORSA plates. Table 1 summarizes the results of the comparison of the ORSA, C_MRSA, MRSA_ID, and TSB-ORSA plates. Of 366 swabs, 30 swabs yielded strains of MRSA (confirmed to be MRSA by *mecA* and *nuc* PCRs) on one or more media within 48 h of incubation. After 22 to 24 h, 17, 18, 20, and 21 MRSA strains were recovered on ORSA, MRSA_ID, C_MRSA, and TSB-ORSA plates, respectively, whereas after 48 h, 23 MRSA strains were recovered on ORSA and MRSA_ID plates and 22 MRSA strains were recovered on C_MRSA and TSB-ORSA plates. As a result, the sensitivities after 24 h were 57% for ORSA plates, 60% for MRSA_ID plates, 67% for C_MRSA plates, and 70% for TSB-ORSA plates. After 48 h, the sensitivities were 77% for ORSA and MRSA_ID plates and 73% for C_MRSA and TSB-ORSA plates. The seven strains that were not recovered on MRSA_ID plates were all isolated in small numbers on ORSA or C_MRSA plates. Similarly, the eight strains that were not detected on C_MRSA plates were present in small numbers on MRSA_ID and ORSA plates.

Whereas the number of true positives was rather similar for all methods examined, more pronounced differences regarding the number of false positives were found. Most FP results were found on ORSA plates (25 FP results after 22 to 24 h and 58 FP results after 48 h) and TSB-ORSA (46 FP results after 48 h and 64 FP results after 72 h). None of the strains with FP results recovered on ORSA plates agglutinated by the conventional_Pastorex technique, nor were they coagulase positive. Eight of 64 strains with FP results recovered on TSB-ORSA media proved to be methicillin-sensitive *S. aureus* (positive by the conventional_Pastorex agglutination test and positive by the tube coagulase test but sensitive to oxacillin and cefoxitin), whereas the 56 remaining false-positive strains did not agglutinate the Pastorex reagent (by the conventional technique) and were coagulase negative. The specificities of ORSA were 92% after 22 to 24 h and 83% after 48 h, whereas the specificities of TSB-ORSA were 86% after 22 to 24 h and 81% after 48 h. As shown in Table 1, the specificities of MRSA_ID and C_MRSA were superior to those of the other media. Importantly, however, in contrast to the manufacturing protocol, FP results were found after 22 to 24 h on MRSA_ID plates (six FP results consisting of two *Enterobacter cloacae* strains, one *Enterobacter aerogenes* strain, and three isolates of CoNS) as well as on C_MRSA media (six FP results consisting of three streptococci, two *Corynebacterium* spp., and one isolate of CoNS). After 48 h, 14 additional FP results were found with MRSA_ID and 28 additional FP results were found with C_MRSA (Table 1). The false-positive results at 48 h were caused by gram-negative rods (*Stenotrophomonas maltophilia*; $n = 3$) and CoNS ($n = 11$) on MRSA_ID plates and by *Corynebacterium* spp. ($n = 10$), CoNS ($n = 15$), gram-negative rods (*Stenotrophomonas maltophilia*; $n = 1$), and streptococci ($n = 2$) on C_MRSA plates.

The possibility of false-positive results on the different agar media requires the performance of additional confirmatory tests before the presence of MRSA is reported. Since the latex agglutination test can be done rapidly and does not increase the time necessary to report the results, we chose to use this confirmatory test in our study. Unfortunately, due to clotting of the colonies, latex agglutination tests cannot be performed with colonies taken directly from ORSA plates. Therefore, in this study, we evaluated whether the Pastorex Staph-Plus agglutination test can be performed with colonies taken directly from MRSA_ID and C_MRSA plates.

The direct_Pastorex agglutination test with colonies from MRSA_ID plates and the conventional_Pastorex agglutination test with colonies from blood agar subcultures from MRSA_ID plates were performed for the 21 true-positive MRSA strains that were isolated (and that were confirmed to be MRSA by *mecA* and *nuc* PCRs) and 18 FP strains. Due to the small number of colonies, no direct_Pastorex agglutination test was done for the two remaining MRSA strains and the two remaining false-positive strains. For all MRSA samples tested, the direct_Pastorex and conventional_Pastorex agglutination tests gave identical results. For 20 of 21 MRSA isolates, both agglutination techniques confirmed the presence of an MRSA strain. One strain positive by the *mecA* and *nuc* PCRs, whether it was taken from MRSA_ID plates or blood agar subcultures, did not agglutinate with the Pastorex agglutination reagent.

Whenever a CoNS caused a false-positive result on MRSA_ID plates, the direct_Pastorex agglutination test as well as the conventional_Pastorex agglutination test results were negative and therefore corrected the false-positive result. In the case of gram-negative rods, positive and negative agglutination reactions were found more frequently by the direct_Pastorex agglutination test than by the conventional_Pastorex agglutination test (three of six false-positive strains agglutinated by the direct_Pastorex agglutination test, whereas one of six false-positive strains agglutinated by the conventional_Pastorex agglutination test), indicating that positive test results should be supplemented by Gram staining. Since the direct_Pastorex agglutination reaction can identify the majority of false-positive MRSA_ID results, the use of MRSA_ID combined with the direct_Pastorex agglutination test increased the specificities to 99% after 22 to 24 h (versus 98% for MRSA_ID without direct_Pastorex agglutination test) and to 98% after 48 h (versus 94% for MRSA_ID without the direct_Pastorex agglutination test). Unmasking of false-positive direct_Pastorex_agglutination reactions due to gram-negative rods by doing a Gram stain further increased the specificities to 100% after 22 to 24 h and to 99.4% after 48 h.

In the case of C_MRSA, direct_Pastorex and conventional_Pastorex agglutination tests were performed with 20 true-positive MRSA strains (which were confirmed to be positive by *mecA* and *nuc* PCRs) and 20 FP strains. Due to the small number of colonies, no direct_Pastorex agglutination test was done with the remaining strains (2 true-positive strains and 14 FP strains). The direct_Pastorex agglutination test confirmed the results for only 15 of 20 true-positive strains isolated on C_MRSA plates. In contrast, the conventional_Pastorex agglutination test confirmed the presence of 19 of 20 true-positive strains. One strain positive by the *mecA* and *nuc* PCRs, whether it was taken from C_MRSA plates or blood agar subcultures, did not agglutinate with the Pastorex agglutination reagent. Therefore, the sensitivity of the direct_Pastorex agglutination test appears to be less than the sensitivity of the conventional_Pastorex agglutination test.

The direct_Pastorex agglutination test was able to correct the results for all strains with false-positive results ($n = 20$) recovered with C_MRSA. The conventional_Pastorex agglutination test result was not interpretable for one gram-negative rod.

In our study, the combined use of C_MRSA with the direct_Pastorex agglutination test increased the specificities from 98 to 98.8% after 22 to 24 h and from 90 to 95.8% after 48 h compared to the specificities achieved by the use of MRSA_ID alone. The addition of Gram staining did not further improve the specificity.

As evidenced by the data in Table 2, strategies in which the direct_Pastorex agglutination test is combined with either MRSA_ID or C_MRSA allow reporting of the majority of MRSA strains (69.6% for MRSA_ID and 63.6% for C_MRSA) within 24 h. Due to an insufficient number of colonies, the reporting time increased from 24 h for two MRSA strains detected with MRSA_ID as well as two MRSA strains

TABLE 2. Cumulative processing times by use of ORSA, MRSA_ID, C_MRSA, and TSB plates and reporting of a confirmed MRSA: comparison of different strategies[a]

Strategy	No. (%) of strains detected at:			
	24 h	48 h	72 h	>96 h
ORSA + conventional_Pastorex		17 (73.9)	23 (100)	
MRSA_ID + direct_Pastorex + Gram staining	16 (69.6)	21 (91.3)	23 (100)	
C_MRSA + direct_Pastorex + Gram staining	14 (63.6)	20 (90.9)	22 (100)	
TSB ORSA + conventional_Pastorex			21 (95.5)	22 (100)

[a] The earliest time point at which the result of any confirmatory test that confirmed the presence of an MRSA strain was known was used to calculate the processing time. The number of strains detected as well as the cumulative percentage is given for four different time points (24 h, 48 h, 72 h, and >96 h).

detected with C_MRSA. Four strains found on C_MRSA plates failed to agglutinate in the direct_Pastorex agglutination reaction; but when the strains were recovered from a blood agar subculture, they agglutinated in the conventional_Pastorex agglutination reaction, thereby increasing the time before reporting with an additional 24 h. In addition, the direct and the conventional_Pastorex agglutination reactions failed for one MRSA strain detected on MRSA_ID and C_MRSA plates. Therefore, additional confirmatory tests (antibiogram tests and *mecA* and *nuc* PCRs) were required for this strain, which resulted in more time delay. The performance of confirmatory tests with presumptive MRSA isolates on ORSA plates by a conventional_Pastorex agglutination test required blood agar subculture. Therefore, although the sensitivity of ORSA plates after 24 h was rather similar to those of MRSA_ID and C_MRSA plates (Table 1), in general, an additional 24 h was required before confirmatory testing was accomplished (Table 2). Due to the time required for the enrichment protocol, 95.5% of the positive results for MRSA obtained by the TSB-ORSA method could be reported only after 72 h (Table 2).

DISCUSSION

For this evaluation, we chose to dilute our samples in order to deliver equivalent inocula to all media. By the use of our study protocol, the sensitivities of all agars tested were similar after 48 h. Recently, Perry et al. also compared the performance characteristics of ORSA, MRSA_ID, and C_MRSA using diluted sample material (6). In accordance with our findings, they reported sensitivities of 62% after 24 h and 78% after 48 h for ORSA (in our study the sensitivities were 57% and 77%, respectively) and a sensitivity of 72% after 48 h for C_MRSA (73% in our study). In their hands, MRSA_ID showed superior sensitivities after 24 h and 48 h compared to those of other media. Our findings could not confirm these results. In real practice, swabs are used to inoculate chromogenic agars directly. Therefore, the sensitivities in real practice might be higher than the sensitivities observed in our comparative study with diluted sample material.

We have observed that whenever strains were not recovered on C_MRSA or MRSA_ID plates, low number of colonies were present on other agars (except TSB-ORSA). Similar observations have been made by others (6). In contrast, the sensitivities of ORSA (57% after 24 h and 77% after 48 h) tended to be higher in our study than the sensitivities reported by Apfalter et al. (51% after 24 h and 69% after 48 h) (1) and Blanc et al. (38% after 48 h) (2).

MRSA_ID and C_MRSA displayed substantially better specificities after 24 h of incubation than ORSA and TSB-ORSA. After 48 h, the specificity of MRSA_ID was superior to that of any other medium tested, resulting in a lower workload with MRSA_ID compared to that required with other agars.

Coagulase-negative staphylococci are a major cause of false-positive results on MRSA_ID and C_MRSA plates. False-positive results due to streptococci and corynebacteria were found only with C_MRSA, whereas *Enterobacter* spp. and *Stenotrophomonas maltophilia* are more likely to cause false-positive results on MRSA_ID plates. The latter finding is not completely surprising, since alpha-glucosidase is also produced by *Stenotrophomonas maltophilia* (3, 5) and the distinction capacity of MRSA_ID is based upon the appearance of a green color due to alpha-glucosidase production. The presence of cefoxitin in the chromogenic medium is apparently inadequate to prevent the growth of certain multiresistant gram-negative rods. According to the manufacturers' guidelines, positive results after 24 h on MRSA_ID and C_MRSA can be reported as true MRSA without additional testing, whereas positive results after 48 h warrant further confirmatory testing. We found a substantial number of false-positive results after 22 to 24 h in both MRSA_ID and C_MRSA media. Therefore, we would recommend confirmatory testing for all samples with positive results at 24 h as well.

To gain time, while waiting for susceptibility testing and subcultures, we wondered whether the agglutination test could be performed directly with colonies taken from chromogenic agars. Our results indicate that the direct_Pastorex agglutination reaction can be done reliably with colonies from MRSA_ID plates. However, some gram-negative rods, whether they are taken directly from MRSA_ID plates or from blood agar subcultures, agglutinate with the reagent as well. A Gram stain can help to circumvent this problem. By combining the direct_Pastorex agglutination test and Gram staining for every presumptive MRSA isolated on MRSA_ID plates, we have shown that almost 70% of true-positive MRSA strains can be reliably reported within 24 h. Although application of the direct_Pastorex agglutination test to presumptive MRSA isolates from C_MRSA plates resulted in a higher number of false-negative results, a similar combination strategy still allowed reporting of 63% of the true-positive MRSA strains within 24 h. Some *S. aureus* strains do not agglutinate with the Pastorex reagents. In order to avoid the reporting of false-negative results, we advise Gram staining of all colored colonies with negative direct_Pastorex agglutination test results, and whenever staphylococci are revealed, we recommend the preparation of a subculture to judge the characteristics of the strain and to perform additional identification tests, if they are required.

In summary, in our laboratory the use of both MRSA_ID and C_MRSA significantly reduced the workload and sped up the processing time compared with those with the use of ORSA. In hospitals like ours, in which large numbers of samples for surveillance cultures are taken from patients in the intensive care unit, where resistant organisms are more prevalent than in other departments, we recommend confirmatory testing of all strains with positive results (even after 24 h).

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