Comparison of the VITEK® 2 System with Conventional Methods for Species Identification and Antimicrobial Susceptibility Pattern of Staphylococcal Carrier Isolates

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Abstract

Staphylococcus aureus, an opportunistic pathogen that inhabits 20% to 70% of human anterior nares and can cause infections, ranging from mild, superficial infections of the skin and soft tissues to severe conditions like sepsis, necrotizing pneumonia and toxic shock syndrome. Healthcare workers are potential asymptomatic nasal carriers, contribute to major infection transmission. The emergence of multidrug-resistant strains necessitates advanced treatment approaches. The study aimed to identify staphylococcal nasal carriers among dental students, and to compare the results obtained from automated identification system, VITEK® 2 with conventional methods for identifying *Staphylococcus* species and determining antibiotic susceptibility patterns. Anterior nasal swabs were collected from 42 study participants were processed as per standard microbiological culture techniques. All the conventionally identified *S. aureus* isolates were subjected to a further analysis using an automated VITEK® 2 SYSTEM. Surprisingly, the automated identification system classified all conventionally proven *S. aureus* isolates as other Staphylococci species *(S. epidermidis* (60%), *S. warneri*, and *S. lentus)*, raising concerns about potential misdiagnosis. Overall, there was a 97.5% categorical agreement between VITEK® 2 system and the reference method, with minimal errors (0.8% very major, 0.6% major, and 1.17% minor). These discrepancies underscore the importance of accurate species identification and highlight the necessity for advanced techniques in infection control strategies, emphasizing the potential impact on decolonization decisions.

Keywords: Categorical Agreement, Conventional-Reference Method, *Staphylococci*, Nasal Carriage, VITEK® 2 system.

Introduction

Staphylococcus aureus, usual colonizers of the anterior nares (20% to 70% of adults), has transformed into a pathogen, posing a potential threat. It is responsible for various surface-level infections and can also lead to deeply entrenched invasive infections. The World Health Organization had classified *S. aureus* under the high-risk pathogen category (priority 2). Increasing incidence of antibiotic resistance (especially methicillin, vancomycin resistance) in *S. aureus* imposes great concern in therapeutic management (1).

Formerly seen as a diverse collection of opportunistic pathogens with diminished virulence, Coagulase-Negative Staphylococci (CoNS) has gained clinical importance in recent decades. Their coexistence with *S. aureus* colonizing the anterior nasal cavity, whether persistently or temporarily, magnifies their significance, making them a potential infectious source. Exposure of CoNS to factors promoting infection plays a major role in the shift of this formerly considered saprophyte into an infection causing agent (2).

Primary Staphylococcal carriers, including those with Methicillin-resistant Staphylococcus *aureus* (MRSA), CoNS species (especially Methicillin-resistant CoNS) among the healthcare workers (HCWs) and their nasal carriage rates also differ depending on their occupation and location. Thus, to minimize transmission, it is

essential to implement periodical surveillance, decolonization measures for carriers, and enhance cross-infection control practices (2). Conventional method that is routinely adopted for species identification of Staphylococci colonizing the anterior nares, include the bacteriological culture on differential and selective media including MacConkey agar, mannitol salt Agar followed by biochemical identification of the species. Also, Kirby Bauer Disc Diffusion method is employed for antimicrobial susceptibility testing (AST). However, these conventional methods are time-consuming and subjective. The turnaround time using these conventional methods is around 48 hours, leading to potential delays in initiating appropriate antibiotic therapy. The Vitek 2 system is an automated microbial identification and AST platform used in clinical microbiology laboratories. It utilizes advanced technology to rapidly identify bacterial and fungal pathogens and determine their susceptibility to various antibiotics. The system employs a combination of biochemical tests, spectrophotometric analysis, and database matching algorithms to generate accurate and reliable results. By streamlining and automating the testing process, the Vitek 2 system enhances laboratory efficiency, improves turnaround times for critical patient samples, and aids healthcare providers in making informed treatment decisions. Nevertheless, the VITEK® 2 SYSTEM has limitations in identifying certain bacteria, particularly Gram-positive cocci (2-4). This study aimed to investigate the frequency of *S.aureus* nasal carriage among dental students along with comparison to the results obtained from automated identification system like VITEK® 2 with conventional methods for identifying *Staphylococcus* species and determining antibiotic susceptibility pattern.

Methodology

Study Protocol and Location

This cross-sectional investigation was carried out for 6 months duration (June to December 2021) at a private teaching dental hospital located at Chennai, Tamil Nadu, India.

Sample Size

A total of forty-two (n=42) dental undergraduates and postgraduates comprising of 18 male and 24 female participants from a private teaching dental hospital were recruited for the study.

Participant Selection Criteria

Dental undergraduates of both sexes, encompassing diabetic and non-diabetic individuals were included as study participants. However, participants with a history of respiratory tract infection / nasal surgery, skin and soft tissue infections, individuals under antimicrobial medications for the past 2 months, and immunocompromised personnel were excluded from the study.

Sample Collection

Anterior nasal samples were collected from the consented participants using sterile cotton swabs pre-moistened in sterile normal saline. The swabs were inserted and gently rotated for 15 seconds in both the nostrils consecutively (2).

The swabs were then subjected to standard microbiological identification methods.

Conventional Identification of *S. aureus*

Aseptically collected nasal swabs were inoculated onto culture media, Mannitol salt agar and MacConkey Agar (incorporated with 0.5% NaCl) (HiMedia Laboratories Pvt Ltd,Mumbai, India). Conventional tests *viz*., Gram staining, oxidase, catalase, O-F fermentation, coagulase (bound and free) production was performed to confirm the species.

Sensitivity to Antimicrobials

Antimicrobial sensitivity of the S. *aureus* strains was evaluated using Kirby Bauer Disc Diffusion method in accordance with CLSI guidelines (4). Antimicrobials tested were fluoroquinolones (levofloxacin and ciprofloxacin), sulphonamides (co-trimoxazole), macrolide (erythromycin), lincosamide (clindamycin), oxazolidinone (linezolid), glycylcycline drug (tigecycline) and antibiotics like nitrofurantoin and tetracycline. Mupirocin sensitivity was determined using an antibiotic disc with 200 µg/disc concentration, and vancomycin sensitivity was determined using an antibiotic disc (6 µg/mL) using the disc diffusion

method. While mecA-mediated oxacillin (methicillin) resistance among strains was determined using the cefoxitin antibiotic disc. D test using clindamycin and erythromycin antibiotic discs were performed to identify inducible clindamycin resistance among isolates. Straightening of the zone of inhibition around the clindamycin disc kept adjacent to the erythromycin disc indicates a positive result. Also a working control of ATCC 25923 strain of *S. aureus* was also included.

Comparative Analysis

In addition, for comparative analysis, isolates of *S. aureus* confirmed phenotypically as carriers were subjected to species identification through the automated system- VITEK® 2, utilizing Staphylococci specific fluorimetric card such as ID-GPC and susceptibility testing using P628 panel. Variance in the results obtained was then analyzed to derive definitive conclusions.

Determination of Antimicrobial Sensitivity Patterns

The analysis of antibiotic susceptibility testing (AST) can lead to two outcomes: (I) Categorical agreement (CA) or (II) Discrepancies/errors. The bacterial isolate's AST pattern was deduced following CLSI interpretation protocol and were designated as either susceptible(S), intermediate (I), or resistant (R) (5, 6). Conversely, discrepancies in AST results were assessed using the scoring pattern indicated in Table 1.

Table 1: Scoring Pattern for Discrepancies in AST Results (7)

Type of Error	Reference/Routine Method (Disc diffusion Method)	Test/Automated method (VITEK® 2)
Very Major Errors (VME)	Resistant	Susceptible
Major Errors (ME)	Susceptible	Resistant
	Susceptible / Resistant	Intermediately susceptible
Minor Errors (mE)	Intermediately susceptible	Susceptible / Resistant

Data Analysis

Statistical analysis was done using IBM SPSS 22. (SPSS Inc, Chicago, USA). Diagnostic measures such as sensitivity, specificity and positive and negative predictive values of VITEK® 2 and conventional method were computed. The Chi-square test was adopted to assess the statistical significance, for evaluating association and levels of concordance of the data respectively. The significance level of the tests (P value) was considered < 0.05.

Results

Comparative Analysis of Bacterial Identification Methods

Of the nasal swabs that were collected from 42 students and processed, 53 isolates of Staphylococci were identified. Among the 53 isolates, 8 (15.09%) were proved as S. *aureus* species when tested using a routine identification method, that is those produced yellow-colored colonies on mannitol salt agar and gave positive results for the presence of

both bound as well as free coagulase when tested using slide and tube coagulase tests respectively. However, when subjected to identification using the VITEK® 2 SYSTEM, all conventionally proven S. *aureus* isolates were determined as CoNS species, namely six (75%) S. *epidermidis* isolates, followed byone (12.5%) S. *warneri* isolate and one (12.5%) S. *lentusisolate*. This demonstrated complete concordance (100%) in genus identification, but no agreement (0%) in species identification when employing the automated method.

Turn Around Time (VITEK® 2)

Identification of *S. epidermidis* isolates was comparatively faster (within 5.82 hrs), while it took 5.83hrs and 5.60 hrs for the identification of *S. warneri* and *S. lentus* isolates respectively.

Analysis of AST Results

As per Clinical and Laboratory Standards Institute (CLSI) recommendation, screening method that is routinely being employed by diagnostic laboratories for MRSA detection includes the Kirby Bauer Disc diffusion method utilizing cefoxitin as the surrogate marker to detect mecA-mediated oxacillin

Table 2: VITEK® 2 SYSTEM- Speciation and Minimum inhibitory concentration (MIC) report

Isolate Identification Number	NCS22		NCS23		NCS24*		NCS42		NCS 47*		NCS 48*		NCS53		NCS54		n=8
Staphylo-coccal sps	*S. warneri*		*S. epidermidis*		*S. epidermidis*		*S. epidermidis*		*S. epidermidis*		*S. epidermidis*		*S. lentus*		*S. epidermidis*		R%
β-lactamase enzyme production	P	+	P	+	P	+	P	+	P	+	P	+	P	+	P	+	100
CX	P	+	P	+	**N**	-	P	+	**N**	-	**N**	-	P	+	P	+	62.5
PenG	≥ 0.5	R	≥ 0.5	R	≥ 0.5	R	≥0.5	R	≥ 0.5	R	≥ 0.5	R	≥ 0.5	R	≥ 0.5	R	100
AMP		R		R		R		R		R		R		R		R	100
OXA	≥ 4	R	≥ 4	R	**≤0.25**	**S**	≥ 4	R	**≤0.25**	**S**	**≤0.25**	**S**	≥ 4	R	≥ 4	R	62.5
GEN	≤ 0.5	S	≥ 16	R	≤ 0.5	S	≥ 16	R	≤ 0.5	S	≤ 0.5	S	≥ 16	R	≤ 0.5	S	37.5
CIP	≤ 0.5	S	≥ 8	R	≥ 8	R	≥ 8	R	≥ 8	R	≥ 8	R	≥ 8	R	≤ 0.5	S	75
LEV	0.5	S	4	I	4	I	≥ 8	R	4	I	4	I	4	I	0.5	S	75
ERY	0.5	S	≥ 8	R	≤0.25	S	≥ 8	R	≤0.25	S	≤0.25	S	≥ 8	R	≤0.25	S	37.5
CLI	0.5	S	0.25	S	≤0.12	S	0.25	S	≤0.12	S	≤0.12	S	0.25	S	0.5	S	0
LZ	1	S	4	S	≤0.5	S	≥ 8	R	≤0.5	S	≤0.5	S	4	S	1	S	12.5
TEI	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S	2	S	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S	≤ 0.5	S	0
VAN	≤ 0.5	S	2	S	≤ 0.5	S	1	S	≤ 0.5	S	≤ 0.5	S	2	S	≤ 0.5	S	0
TET	≥16	R	1	S	≤ 1	S	1	S	1	S	≤ 1	S	≤ 1	S	≥16	R	25
RIF	2	I	2	S	≤0.03	S	≤0.03	S	≤0.03	S	≤0.03	S	≤0.03	S	2	I	25
TGC	≤0.12	S	≤0.12	S	≤0.12	S	0.25	S	≤0.12	S	≤0.12	S	≤0.12	S	≤0.12	S	0
COT	≤ 10	S	≥ 320	R	≤ 10	S	≥ 320	R	≤ 10	S	≤ 10	S	≥ 320	R	≤ 10	S	37.5

* MRSA by Kirby Bauer Method However MSSA by VITEK® 2 SYSTEM

(CX- Cefoxitin, PenG- Benzylpenicillin, AMP-Ampicillin, OXA- Oxacillin, GEN- Gentamicin, CIP- Ciprofloxacin, LEV- Levofloxacin, ERY- Erythromycin, CLI- Clindamycin, LZ-Linezolid, TEI- Teicoplanin, VAN- Vancomycin, TET- Tetracycline, RIF- Rifampicin, TGC- Tigecycline, COT- Cotrimoxazole)

resistance. Cefoxitin is being recommended over oxacillin primarily to a triad of advantages, non-requirement of altered incubation temperature, no specific ingredient/medium, is a better inducer of mecA regulatory system. Based on cefoxitin susceptibility results, all the tested *S. aureus* isolates (n=8) were scored as MRSA isolates. However, when these same isolates were tested using VITEK®2 System, only 5 out of 8 were proven as MRSA, while the remaining 3 showed susceptibility to cefoxitin, termed as Methicillin-Sensitive *Staphylococcus aureus* (MSSA). Notably, testing for oxacillin resistance using the VITEK®2 indicated that MRSA isolates (n=5) were resistant to oxacillin, while all MSSA isolates (n=3) were susceptible. The automated system (VITEK® 2) detected exclusively *S. epidermidis* isolates that exhibited the observed inconsistency. AST results revealed that all isolates produced the enzyme, β-lactamase thus exhibited resistance to benzyl penicillin and ampicillin. All isolates tested (n=8) exhibited susceptibility to both vancomycin, and tigecycline (Table 2). None of the isolates tested exhibited resistance to high or low-level mupirocin. Among the *S. aureus* isolates tested, 25% exhibited resistance to erythromycin, and 12.5% to co-trimoxazole.

Notably, one *S. warneri* isolate and one *S. lentus* isolate were identified as MR-CoNS. Nevertheless, both exhibited susceptibility to most of the

antibiotics tested, except for tetracycline (resistance) and rifampicin (intermediate susceptibility). Additionally, among *S. epidermidis* isolates, 33.3% showed co-trimoxazole resistance, while 83.33% exhibited fluoroquinolone resistance (ciprofloxacin resistance = 5, levofloxacin resistance = 1, and levofloxacin intermediate resistance = 4).

In accordance with FDA guidelines, the minimal performance requirements for Antibiotic Susceptibility Testing (AST) involve achieving a Categorical Agreement (CA) of over 90%, a Major Error rate < 3%, and a Very Major Error rate of >/ 1.5% (8). Our study demonstrates a complete (100%) CA for vancomycin and tigecycline, followed by 75% CA for antibiotics like clindamycin, co-trimoxazole and tetracycline. However, a lower concordanceof 50% was recorded for erythromycin and least (37.5%) when tested for cefoxitin (Table 3).

Table 3: Contrasting Antibiotic Susceptibility Testing (AST) outcomes for Coagulase-Negative Staphylococci (CoNS) between the test and reference methods

	Disc Diffusion Method (Reference Method)			VITEK® 2 SYSTEM (Test Method)			Discrepancies Errors (n)			Categorical Agreement
	S	I	R	S	I	R	VME	ME	mE	CA%
CX	0	0	8	3	0	5	3	0	0	37.5
TGC	8	0	0	8	0	0	0	0	0	100
VAN	8	0	0	8	0	0	0	0	0	100
TET	6	2	0	6	0	2	0	0	2	75
CLI	6	2	0	8	0	0	0	0	2	75
COT	7	0	1	5	0	3	0	2	0	75
ERY	4	2	2	5	0	3	1	1	2	50
TOTAL	39	6	11	43	0	13	4	3	6	-
INCIDENCE RATE	69.7%	10.7%	19.6%	46.8%	0%	23.2%	0.8%	0.6%	1.17%	97.5%

*VME- Very major error, ME- Major error, mE- Minor error
(CX- Cefoxitin, TGC - Tigecycline, VAN- Vancomycin, TET-Tetracycline, CLI- Clindamycin, COT- Cotrimoxazole, ERY-Erythromycin)

Four very major errors were identified, three associated with cefoxitin, and one with erythromycin.

The occurrence of three very major errors (VMEs) with cefoxitin can be linked to differences in CLSI-MIC breakpoints between S. *aureus* and CoNS. The automated system-VITEK® 2 initially misinterpreted all three strains labeled as cefoxitin-negative to be S. epidermidis, despite routine identification confirming them to be S. *aureus.*

Comparing antibiotic Susceptibility Testing results from Disk Diffusion and the VITEK® 2 System showed less than 95% categorical agreement across all isolates. Notably, Categorical Agreement was lower (75%), specifically for antimicrobials such as Clindamycin (lincosamide), Tetracycline (antibiotic) and Co-trimoxazole (sulphonamide). Erythromycin had the least categorical agreement (50%), followed by Cefoxitin at 37.5%. Cefoxitin displayed a majority of three Very Major Errors (VMEs), while Co-trimoxazole had two Major Errors (MEs), and Erythromycin had one Major Error. Two Minor Errors were observed for each of Erythromycin, Tetracycline, and Clindamycin.

The comprehensive agreement in susceptibility categories between the automated system-VITEK® 2 and the Disk Diffusion technique in this study stood at 97.5%. The percentages of VME, ME, mE were recorded as 0.8%, 0.6%, and 1.17%, respectively.

The results of the current study showed that all S. *aureus* isolates confirmed conventionally were incorrectly identified as CoNS (other non-pathogenic Staphylococcus species) when tested using automated identification system-VITEK® 2. In particular, S. epidermidis was detected in three samples (60%). Meanwhile, S. warneri and S. lentus were each detected in one sample, constituting 20%.

Discussion

Co-existence of *S.aureus* with other saprophytic bacteria in the human body may lead to various infections (9). Persistent *S. aureus* presence in human nasal passages heightens predisposition to infection, especially in immunocompromised individuals (10-14). Healthcare professionals, including dental students, are significant reservoirs for transmission of staphylococcal infections owing to a prolonged and continual harbor of the bacterium in their anterior nares acquired during hospital internships (3).

A timely acquisition of the pathogen's antibiotic susceptibility pattern is crucial for an effective infection control protocol. The current bacterial identification procedures cause delays, which impact patient health by prolonging the administration of empirical medications and hindering timely treatment. Newer technologies are being developed for efficient use in laboratories to reduce the workload and deliver faster results (15).

Since an elevated incidence of *S. aureus* infections and the prevalence of multidrug-resistant strains necessitate a quicker, more accurate identification module, modern techniques and instruments for bacterial identification in microbiological laboratories offer dual benefits in clinical and financial aspects (7, 16). The VITEK® 2 System, evolving technologies from 1970s, employs photometers and calorimeters integrated to interpret bacterial growth. This information is presented graphically for ease of interpretation (17).

Although the VITEK® 2 System offers swift results and minimizes Turnaround Time (TAT), conventional methods are deemed for enhanced precision and reliability. The present study observed that the automated system (VITEK®2) misidentified conventionally confirmed pathogenic S. *aureus* strains as other non-pathogenic Staphylococcus species. These findings are consistent with precursory reports stating the misidentification rates of S. *aureus* ranging between 96% -98.8% (7, 18).

In our study, we recorded an average turnaround time of 5.75 hours, which compares favorably to the normal antibiotic susceptibility testing time (NAST) of 48 hours. This value aligns closely with the values reported by Barenfanger*et al.* (16) stating a turnaround time of 5.2hrs when compared with NAST duration of 44.5hrs.

Our study demonstrated 100% categorical agreement for vancomycin which is in line with reports by Ligozzi *et al.* (7) However, they reported a discrepancy for teicoplanin antibiotic (4 of 22) teicoplanin resistant strains were misinterpreted as sensitive to the drug.

A comparative analysis between the VITEK® 2 System and Disk Diffusion technique revealed less than 95% Categorical Agreement across all isolates. Notably, clindamycin, tetracycline, and co-trimoxazole exhibited a lower Categorical Agreement at 75%, while erythromycin and cefoxitin demonstrated even lower rates at 50% and 37.5%, respectively. Major Errors were observed for erythromycin and co-trimoxazole, with cefoxitin showing a majority of Very Major Errors. While the VITEK® 2 System demonstrated high overall concordance with the Disk Diffusion technique, disparities were evident in specific antibiotics, notably cefoxitin and erythromycin. Understanding the sources of errors, especially the impact of different breakpoints, is crucial for improving the interpretive accuracy of automated systems in Antibiotic Susceptibility Testing.

Table 4 demonstrates a comparative analysis of error rates and categorical agreement between the Disk diffusion method and the Vitek method across the studies (19, 20).

Table 4: Analysis of error rates and categorical agreement between the Disk diffusion method and the Vitek method across the studies

Study	Method	Very Major Error (VME)	Major Error (ME)	Minor Error (mE)	Categorical Agreement (CA)
Jorgensen *et al*	Disk diffusion	0%	0%	2.2%	97.8%
	VITEK® 2	0.6%	0%	2%	97.8%
Mücahide EK *et al*	Disk diffusion	1.4%	0.9%	0.5%	97.2%
	VITEK® 2	2.6%	1.5%	0.5%	95.4%
Present study	Disk diffusion	0%	0.89%	1.07%	89.13%
	VITEK® 2	0.8%	0.6%	1.17%	97.5%

In general, the very major error rates for both methods (conventional and automated) are relatively low across all studies, with Jorgensen *et al* reporting the lowest rates for both methods. Major errors are either absent or minimal in most cases. Jorgensen *et al* (19) and Present study report no major errors for both methods. The minor error rates vary across studies and methods. Jorgensen *et al* and Mücahide EK *et al* report relatively low rates for both methods, while Present study reports slightly higher rates, especially with the Disk diffusion method (19, 20).

Categorical agreement rates are generally high across all studies and methods. Jorgensen et al consistently report high agreement rates for both methods. Mücahide EK et al (19, 20) and Present study show slightly lower agreement rates, especially with the Disk diffusion method in the Present study. Overall, while the Vitek method generally shows lower error rates and higher categorical agreement compared to the Disk diffusion method, the specific performance varies across studies. Jorgensen et al (19) consistently demonstrate high performance with both methods, while the Present study shows relatively lower performance, especially with the Disk diffusion method. Mücahide EK et al (20) fall in between, with slightly lower performance compared to Jorgensen et al (19).

While the VITEK® 2 System demonstrated high overall concordance with the Disk Diffusion technique, disparities were evident in specific antibiotics, notably cefoxitin and erythromycin. Understanding the sources of errors, especially the impact of different breakpoints, is crucial for improving the interpretive accuracy of automated systems in antibiotic Susceptibility Testing.

In conclusion, the detection of MRSA in the nasal passages of dental students poses a significant concern for potential transmission within the student community and beyond. While the automated VITEK® 2 SYSTEM is recognized for its efficiency in identifying bacteria, our study reveals limitations in its accuracy, particularly in distinguishing between Staphylococcus *aureus* and other non-pathogenic species. This disparity could lead to misguided antibiotic prescriptions, undermining treatment protocols. While there is a high agreement in susceptibility categories, the presence of inaccuracies underscores the necessity for further investigation to ensure precise identification and antibiotic prescribing practices. Addressing these shortcomings is crucial for enhancing patient care and combating the spread of MRSA.

Conclusion

Prompt and accurate microbiological testing significantly improves patient care and promotes antibiotic stewardship. It allows for targeted treatment, reduces reliance on broad-spectrum antibiotics, and optimizes antibiotic selection. By facilitating early diagnosis and tailored therapy, it speeds up recovery, prevents complications, and limits antibiotic overuse and resistance. Additionally, microbiological testing aids in surveillance of resistance patterns, guiding broader strategies to combat antibiotic resistance. Overall, it plays a crucial role in improving outcomes,

minimizing unnecessary antibiotic exposure, and preserving antibiotic effectiveness.

Abbreviation

Clinical and Laboratory Standards Institute (CLSI); American Type Culture Collection (ATCC); Food and Drug Administration (FDA)

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Conflict of Interests

The authors assert that no conflicts of interest exist.

Ethics Approval

The Institutional Ethics Committee at Sree Balaji Dental College and Hospital, BIHER, Chennai scrutinized and approved the study protocol (Reference number SBDCH/IEC/06/2021/1).

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