In vitro Antimicrobial Susceptibility of *Staphylococcus pseudintermedius* isolates of human and animal origin

Romney M. Humphries[1*], Max T. Wu[2], Lars F. Westblade[3], Amy E. Robertson[4], Carey-Ann D. Burnham[5], Meghan A. Wallace[5], Eileen M. Burd[6,7], Sara Lawhon[8], and Janet A. Hindler[1]

[1]UCLA David Geffen School of Medicine, Los Angeles, CA

[2]Landstuhl Regional Medical Center, Landstuhl, Deutschland

[3]Weill Cornell Medical College, New York, NY

[4] New-York Presbyterian Hospital, Weill Cornell Medical Center, New York, NY

[5]Washington University School of Medicine, St. Louis, MO

[6] Emory University School of Medicine, Atlanta, GA

[7] Emory Antibiotic Resistance Center, Atlanta, GA

[7]Vetrinary Medicine and Biomedical Sciences, Texas A&M University, TX

*Corresponding author

Mailing address: 10833 Le Conte Avenue. Brentwood Annex, Los Angeles, CA 90095.

Email: rhumphries@mednet.ucla.edu

ABSTRACT

Minimum inhibitory concentration (MIC) results for 115 Staphylococcus intermedius group isolates are presented. 33% were methicillin resistant, among which 51.4% were susceptible to doxycycline, 29.7% to clindamycin and 21.6% to trimethoprim-sulfamethoxazole. All isolates were susceptible to ceftaroline, daptomycin, linezolid, nitrofurantoin, quinupristin-dalfopristin, rifampin, tigecycline, and vancomycin. 82.6%, 67.8% and 23.5% of all isolates were susceptible to ciprofloxacin, erythromycin, and penicillin. No isolates harbored mupA or qacA/B genes, suggestive of no resistance to mupirocin or chlorhexidine.

TEXT

The *Staphylococcus intermedius* group (SIG) is comprised of *Staphylococcus intermedius, Staphylococcus pseudintermedius*, and *Staphylococcus delphini.* These Gram-positive cocci are tube coagulase positive and slide coagulase negative (except *S. intermedius*), and may be misidentified as *Staphylococcus aureus* by clinical laboratories that test human specimens (1). A colonizer of the nares and anal mucosa of cats and dogs, the presence of *S. pseudintermedius* is increasingly being recognized in human diagnostic specimens (2). This may in part be due to improved diagnostic technologies, such as matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) now being used in many clinical laboratories. *S. pseudintermedius* have been documented to cause invasive infections in humans, including brain abscesses, endocarditis, and bacteremia (3). Methicillin resistance among *S. pseudintermedius* isolated from dogs is increasing (4), with rates of up to 47% in some regions of the world (5). This resistance is predominantly due to the dissemination of the ST71 clonal lineage in Europe and ST68 clonal lineage in North America (4). Methicillin resistant (MR) isolates often display

resistance to other classes of antimicrobials used in veterinary medicine, including aminoglycosides, fluoroquinolones, lincosamides, macrolides, tetracyclines and also to chloramphenicol and trimethoprim-sulfamethoxazole (SXT) (6). However, there are limited susceptibility data available for *S. pseudintermedius* with antimicrobials used for humans. We recently conducted a study to evaluate oxacillin and cefoxitin disk and minimum inhibitory concentration (MIC) results as predictors of methicillin resistance (encoded by *mecA*) in a collection of 115 SIG isolated from human and veterinary specimens associated with clinical infections. This study documented that cefoxitin testing, which is recommended by the Clinical and Laboratories Standards Institute (CLSI) to predict methicillin resistance for other species of staphylococci, is a poor predictor of *mecA* in SIG, whereas both oxacillin disk and MIC tests accurately detect *mecA*-mediated oxacillin resistance in these isolates (7). As a result of our study, CLSI published *S. pseudintermedius*-specific oxacillin breakpoints in the 26th edition of the M100S standard (8). The present study documents the results of antimicrobial susceptibility testing (AST) for this collection of 115 SIG isolates, including 111 isolates of *S. pseudintermedius* (45 from human, 56 from canine, 7 from feline, 2 from avian and 1 from porcine sources) and 4 isolates of *S. delphini* (3 from equine and 1 from avian sources).

Bacterial isolates were described in our previous article (7). AST was performed according to the CLSI reference broth microdilution MIC method (8), using panels prepared in-house with cation-adjusted Mueller Hinton Broth (MHB). MHB was supplemented with 50 mg/L $CaCl_2$ for daptomycin testing and 2% NaCl for oxacillin testing (9). Fifteen antimicrobial agents were tested (Table 1). BMD tests were read following 16-20 hours incubation at 35°C in ambient air for all antimicrobials except oxacillin and vancomycin, where the final reading was done following 24 hours' incubation. MIC results were interpreted according to *Staphylococcus* spp. breakpoints listed in CLSI M100S 26th edition,

Journal of Clinical Microbiology

including use of the new oxacillin *S. pseudintermedius* breakpoints and ceftaroline and vancomycin breakpoints for *S. aureus* (8). Because there are no CLSI tigecycline breakpoints, the Food and Drug Administration (FDA) breakpoint for *S. aureus* was used. All isolates with penicillin-susceptible MICs (≤0.12 µg/ml) were also tested by penicillin disk diffusion using the standard CLSI method and examined for beta-lactamase production using a BBL Cefinase™ disk (BD, Sparks MD). In addition to taking zone measurements, the zone edges were evaluated for sharp versus fuzzy borders around the penicillin disks. Beta-lactamase testing was performed using growth taken from the zone margin surrounding a penicillin disk test on BBL Mueller Hinton agar (MHA, BD) after 16-18 hours' incubation. *mecA* PCR and SCC*mec* typing was performed as described in our previous article (7). Mupirocin resistance was determined by PCR for the *mupA* gene and chlorhexidine resistance by PCR for the *qacA/B* gene, as described elsewhere (10).

MIC results obtained for the 115 isolates are shown in Table 1. Thirty-seven isolates (32.2%) harbored the *mecA* gene, including 4 of human origin and 33 of veterinary origin. Using the CLSI M100S 26th edition *Staphylococcus* spp. interpretive criteria, 33 of the 78 (42.3%) *mecA*-negative isolates had penicillin susceptible MICs of ≤0.12 µg/mL (Table 1). For 27/33 isolates, MICs were ≤0.06 µg/ml, penicillin zone measurements were susceptible at ≥29 mm and induced nitrocefin tests were negative. 6/33 (18.2%) yielded a positive induced nitrocefin test, indicating the presence of a beta-lactamase, including 5 human isolates and 1 animal isolate. Six isolates demonstrated penicillin zones ≤28 mm (resistant) and all had "sharp" zone edges. Five of these isolates had penicillin MICs of 0.12 µg/mL and 1 isolate had a penicillin MIC of ≤0.03 µg/mL. Repeat testing in two laboratories confirmed results. When the nitrocefin tests were performed using un-induced colonies (i.e. not from a penicillin zone margin), variable results were obtained, with 0-4 of the 6 isolates yielding a positive result in different laboratories, on different days when testing colonies grown on

Journal of Clinical Microbiology

BAP or on MHA. As such, a test for beta-lactamase production should be performed for all penicillin-susceptible *S. pseudintermedius* isolates, as is done for other *Staphylococcus* spp. Whether a penicillin zone edge test is sufficient for this purpose, or if an induced nitrocefin-based test is needed, remains to be determined. However, in our limited analysis, the penicillin zone edge test was 100% concordant with nitrocefin results obtained when testing induced colonies. All isolates were susceptible to ceftaroline, the cephalosporin with high affinity binding to PBP2a expressed by *mecA.*

With regards to the non-beta-lactam agents, significant differences were noted in the percentage of methicillin-resistant isolates susceptible to doxycycline, SXT, and clindamycin, as compared to what has been documented with contemporary isolates of *S. aureus* (11). This constellation of multi-drug resistance is consistent with the multi-drug resistant (MDR) *S. pseudintermedius* clones, ST68 and ST71, which harbor mutations within *gyrA* and *grlA* (conferring resistance to fluoroquinolones), as well as a TN5404-like transposon element that harbors the *dfrG* (sulfamethoxazole resistance) and *ermB* (clindamycin and erythromycin resistance) genes (4). Interestingly, differences were noted in our collection based on the SCC*mec* type. Isolates with SCC*mec* V were more commonly resistant to erythromycin and clindamycin (10/11 isolates, 90.9%), SXT (10/11 isolates, 90.9%), doxycycline (8/11 isolates, 72.7%) and ciprofloxacin (9/11 isolates, 81.8%) as compared to those with SCC*mec* types IV or III. For SCC*mec* type IV, 4/8 (50.0%), 8/8 (100%), 1/8 (12.5%), and 0/8 (0.0%) isolates were resistant to these antimicrobials, respectively. For isolates with SCC*mec* type III, 4/9 (44.4%), 2/9 (22.2%), 4/9 (44.4%) and 0/9 (0.0%) were resistant. Isolates of the MDR North American ST68 lineage harbor SCC*mec* V, similar to the more resistant isolates in our collection (4).

Journal of Clinical Microbiology

Doxycycline susceptibility was 89.7% among *mecA*-negative isolates and only 51.4% among *mecA*-positive isolates (Table 1). This is in striking contrast to doxycycline susceptibility rates among human isolates of methicillin-resistant *S. aureus* (MRSA), which were 96% among a collection of >4,000 isolates recovered from human diagnostic specimens in 2010 (12). Doxycycline susceptibility rates were similarly high among methicillin-resistant CoNS, at 94.1% in one study of 1,473 isolates (13). Our data are consistent with previous studies that documented 31-38% doxycycline susceptibility among methicillin-resistant *S. pseudintermedius* (MRSP) isolates from canine sources (14, 15). No difference was noted in susceptibility to doxycycline between human (n=5, 40.0% susceptible) and veterinary (n=32, 53.1% susceptible) MRSP isolates in the present study.

Of note, canine-specific breakpoints for doxycycline have been proposed to accommodate the pharmacokinetics of doxycycline doses used for dogs. The canine breakpoints are ≤0.125 µg/mL (susceptible), 0.25 µg/mL (intermediate) and ≥0.5 µg/mL (resistant), but these have yet to be published in the CLSI VET antimicrobial susceptibility testing document (16). The lowest concentration of doxycycline tested in our study was 1 µg/mL, and as such we cannot estimate the effect these breakpoints would have on our collection of isolates. However, 35% of *mecA*-positive and 10.2% of *mecA*-negative isolates had MICs of 2 – 4 µg/mL, which are resistant by the canine breakpoints but susceptible by the human breakpoints. Resistance to the tetracyclines is mediated through acquisition of tetracycline resistance genes (*tet* genes), four of which have been identified among *S. pseudintermedius* isolates. These are *tet*(M) and *tet*(O), which mediate ribosomal protection, and *tet*(K) and *tet*(L), which encode efflux pumps. The most commonly occurring of these are *tet*(M) and *tet*(K) in *S. pseudintermedius* (16, 17). Isolates that harbor none of these genes typically have MICs ≤0.125 µg/mL to doxycycline, whereas acquisition of the *tet*(M) gene can be associated with MICs that are elevated, but below the 4 µg/mL CLSI M100S 26th

Journal of Clinical Microbiology

edition susceptible breakpoint. Clinically, it is unclear whether such isolates that are susceptible by the CLSI M100S 26th edition breakpoint and harbor a *tet* gene are associated with treatment failures, but these isolates would be considered resistant by the proposed veterinary breakpoint (16). The EUCAST susceptible breakpoint for doxycycline is ≤1 µg/mL for human isolates of *Staphylococcus* spp. (www.eucast.org) and when applying this breakpoint, only 18.1% of methicillin-resistant and 79.5% of methicillin-susceptible isolates in our study would be doxycycline susceptible. Regardless, the *tet* genes are carried on Tn5801 and Tn916 elements (6), the same as are found in human and veterinary isolates of tetracycline-resistant *S. aureus* (18). The Tn916 *tet*(M) gene was found in all isolates of the clonal complex (CC) 398 of *S. aureus,* suggesting this element was integrated into the genome of the clone early and disseminated vertically. This may also be the case for the ST71 and ST68 clonal lineages of *S. pseudintermedius,* and may account for the common occurrence of doxycycline resistance in these isolates. Doxycycline resistance may also be selected for through the common use of this agent for the treatment of pyoderma in small animal veterinary medicine.

SXT susceptibility was only 21.6% among *mecA* positive isolates. In contrast, human isolates of MRSA are typically susceptible to this agent; in 2013, 98.0% of isolates in a collection of over 9,000 MRSA were susceptible to SXT (19). SXT susceptibility is lower among coagulase-negative staphylococci. In the same study conducted in 2013 52.7% of 2,268 methicillin-resistant coagulase-negative staphylococci were susceptible to SXT (19).

All isolates in this study that were resistant to erythromycin were also resistant to clindamycin and susceptibility rates for both agents were only 29.7% among MRSP (Table 1). Consequently, no inducible clindamycin resistance was observed, although an inducible *erm* gene has been documented previously in *S. pseudintermedius* (20).

Journal of Clinical Microbiology

We documented 51.4% ciprofloxacin susceptibility in MRSP, which is similar to what has been observed for MRSA and MR coagulase-negative *Staphylococcus* (CoNS) isolates (19). However, this susceptibility rate is significantly higher than has been documented in some studies of veterinary SIG isolates, where susceptibility rates as low as 2.7% have been reported using the same susceptible breakpoint of 1 µg/mL (21). A single point mutation in topoisomerase II or IV genes confers fluoroquinolone resistance in *S. pseudintermedius* (22).

All isolates were susceptible to ceftaroline, daptomycin, linezolid, nitrofurantoin, quinupristin-dalfopristin, rifampin, tigecycline, and vancomycin. There are currently no vancomycin breakpoints for the SIG, as the CLSI only publishes *S. aureus* and CoNS breakpoints for this antimicrobial agent. However, unlike the CoNS, where the modal MIC for vancomycin is 2.0 µg/mL, we found vancomycin MIC mode to be 1.0 µg/mL, similar to what is documented for *S. aureus.* As such, it may be reasonable for clinical laboratories to interpret vancomycin MICs using the more conservative *S. aureus* susceptible breakpoints of ≤2.0 µg/mL when SIG is encountered, as compared to the ≤4 µg/mL breakpoint for CoNS in the M100S or for *Staphylococcus* spp. in the VET01, CLSI standards. Similar to what has been seen in other studies of SIG (23) we did not document any cases of high-level mupirocin resistance among the isolates in this collection, nor did we detect the presence of the *qacA/B* gene in any isolates, suggestive of the absence of chlorhexidine resistance in this collection of isolates.

In summary, we present *in vitro* susceptibility results for a large collection of SIG clinical isolates tested by the CLSI reference BMD MIC method. Laboratories should carefully review susceptibility results for all coagulase-positive staphylococci and consider using additional identification procedures, such as MALDI-TOF MS or an automated

Journal of Clinical Microbiology

instrument, for isolates that are doxycycline and/or SXT resistant, a phenotype common to *S. pseudintermedius,* but unusual for *S. aureus.* This is important, as correct identification of these isolates is critical to accurate testing of SIG with oxacillin to detect methicillin resistance. Clinicians should be cognizant of the dramatic difference in SXT, clindamycin, and doxycycline susceptibility between SIG and *S. aureus,* as these agents are commonly prescribed as empiric therapy for MRSA in wound and skin structure infections. While overall, susceptibility to these antimicrobials was higher in human than in animal isolates (Table 1), this is likely due to the significantly higher proportion of *mecA*- positive isolates in the veterinary collection, a bias of our data set. A second limitation of the present study is the inclusion of only 4 *S. delphini* and 0 *S. intermedius* isolates; further data will determine if susceptibility rates differ significantly for these isolates as compared to *S. pseudintermedius.* It is worth noting, however, that *S. intermedius* is very infrequently isolated in veterinary or human clinical laboratories, but rather is a constituent of the normal nares flora of the wild pigeon (24).

Journal of Clinical Microbiology

References

1. **Borjesson S, Gomez-Sanz E, Ekstrom K, Torres C, Gronlund U.** 2015. *Staphylococcus pseudintermedius* can be misdiagnosed as *Staphylococcus aureus* in humans with dog bite wounds. Eur J Clin Microbiol Infect Dis **34**: 839-844.

2. **Lee J, Murray A, Bendall R, Gaze W, Zhang L, Vos M.** 2015. Improved detection of *Staphylococcus intermedius* group in a routine diagnostic laboratory. J Clin Microbiol **53**: 961-963.

3. **Kelesidis T, Tsiodras S**. 2010. *Staphylococcus intermedius* is not only a zoonotic pathogen, but may also cause skin abscesses in humans after exposure to saliva. Int J Infect Dis **14**: e838-841.

4. **Perreten V, Kadlec K, Schwarz S, Gronlund Andersson U, Finn M, Greko C, Moodley A, Kania SA, Frank LA, Bemis DA, Franco A, Iurescia M, Battisti A, Dulm B, Wagenaar JA, van Duijkeren E, Weese JS, Fitzgerald JF, Rossano A, Guardabassi L**. 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an international multicentre study. J Antimicrob Chemother **65**: 1145-1154.

5. **Feng Y, Tian W, Lin D, Luo Q, Zhou Y, Yang T, Deng Y, Liu YH, Liu JH.** 2012. Prevalence and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in pets from South China. Vet Microbiol **160**: 517-524.

6. **McCarthy AJ, Harrison EM, Stanczak-Mrozek K, Leggett B, Waller A, Holmes MA, Lloyd DH, Lindsay JA, Loeffler A**. (2015) Genomic insights into the rapid emergence and evolution of MDR in *Staphylococcus pseudintermedius*. J Antimicrob Chemother **70**: 997-1007.

7. **Wu MT, Burnham CA, Westblade LF, Dien Bard J, Lawhon SD, Wallace MA, Stanley T, Burd E, Hindler J, Humphries RM.** 2015. Evaluation of oxacillin and cefoxitin disk and MIC

breakpoints for the prediction of methicillin resistance in human and veterinary isolates of *Staphylococcus intermedius* group. J Clin Microbiol. In press

8. **CLSI**. 2016. Performance Standards for Antimicrobial Susceptibilty Testing M100S, 26th Ed. Wayne, PA: Clinical and Laboratory Standards Institute.

9. **CLSI**. 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, Ninth Edition. Wayne, PA: Clinical and Laboratory Standards Institute.

10. **Fritz SA, Hogan PG, Camins BC, Ainsworth AJ, Patrick C, Martin MS, Krauss MJ, Rodriguez M, Burnham CA.** 2013. Mupirocin and chlorhexidine resistance in *Staphylococcus aureus* in patients with community-onset skin and soft tissue infections. Antimicrob Agents Chemother **57**: 559-568.

11. **Richter SS, Diekema DJ, Heilmann KP, Dohrn CL, Crispell EK, Riahi F, McDanel JS, Satola SW, Doern GV.** 2014. Activities of vancomycin, ceftaroline, and mupirocin against *Staphylococcus aureus* isolates collected in a 2011 national surveillance study in the United States. Antimicrob Agents Chemother **58**: 740-745.

12. **Jones RN, Stilwell MG, Wilson ML, Mendes RE**. 2013. Contemporary tetracycline susceptibility testing: doxycycline MIC methods and interpretive criteria (CLSI and EUCAST) performance when testing Gram-positive pathogens. Diagn Microbiol Infect Dis **76**: 69-72.

13. **Jones RN, Farrell DJ, Sader HS, Castanheira M.** 2011. Abstr. 21st ECCMID. Abstr. 944.

14. **Weese JS, Sweetman K, Edson H, Rousseau J**. 2013. Evaluation of minocycline susceptibility of methicillin-resistant *Staphylococcus pseudintermedius*. Vet Microbiol **162**: 968-971.

15. **Hnot ML, Cole LK, Lorch G, Papich MG, Rajala-Schultz PJ, Daniels JB**. 2015. Evaluation of canine-specific minocycline and doxycycline susceptibility breakpoints for

meticillin-resistant *Staphylococcus pseudintermedius* isolates from dogs. Vet Dermatol **26**: 334-e371.

16. **Maaland MG, Papich MG, Turnidge J, Guardabassi L**. 2013. Pharmacodynamics of doxycycline and tetracycline against *Staphylococcus pseudintermedius*: proposal of canine-specific breakpoints for doxycycline. J Clin Microbiol **51**: 3547-3554.

17. **Schwarz S, Roberts MC, Werckenthin C, Pang Y, Lange C**. 1998. Tetracycline resistance in *Staphylococcus* spp. from domestic animals. Vet Microbiol **63**: 217-227.

18. **de Vries LE, Christensen H, Skov RL, Aarestrup FM, Agerso Y**. 2009. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in *Staphylococcus aureus* from humans and animals. J Antimicrob Chemother **64**: 490-500.

19. **Sader HS, Flamm RK, Jones RN**. 2013. Antimicrobial activity of ceftaroline tested against staphylococci with reduced susceptibility to linezolid, daptomycin, or vancomycin from U.S. hospitals, 2008 to 2011. Antimicrob Agents Chemother **57**: 3178-3181.

20. **Gold RM, Lawhon SD**. 2013. Incidence of inducible clindamycin resistance in *Staphylococcus pseudintermedius* from dogs. J Clin Microbiol **51**: 4196-4199.

21. **Ruscher C, Lubke-Becker A, Semmler T, Wleklinski CG, Paasch A, Soba A, Stamm I, Kopp P, Wieler LH, Walther B**. 2010. Widespread rapid emergence of a distinct methicillin- and multidrug-resistant *Staphylococcus pseudintermedius* (MRSP) genetic lineage in Europe. Vet Microbiol **144**: 340-346.

22. **Descloux S, Rossano A, Perreten V.** 2008. Characterization of new staphylococcal cassette chromosome mec (SCCmec) and topoisomerase genes in fluoroquinolone- and methicillin-resistant *Staphylococcus pseudintermedius*. J Clin Microbiol **46**: 1818-1823.

23. **Godbeer SM, Gold RM, Lawhon SD**. 2014. Prevalence of mupirocin resistance in *Staphylococcus pseudintermedius*. J Clin Microbiol **52**: 1250-1252.

276 24. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. 20007.
277 Reclassification of phenotypically identified Staphylococcus intermediu strains. J Clin
278 Microbiol. 45:2770-2778.

279
280

281

282 Table 1. MIC values of 15 antimicrobial agents for *Staphylococcus intermedius* group (n=115) when tested by CLSI reference broth

283 microdilution MIC method in CAMHB

Antimicrobial	Number of Isolates at MIC (μg/mL)										% susceptible				
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	human	animal	*mecA*-	*mecA*+	All
Ceftaroline			115[a]								100	100	100	100	100
Ciprofloxacin				94[a]	1			20[b]			91.1	77.1	97.4	51.4	82.6
Clindamycin				78[a]		1			36[b]		80.0	60.0	85.9	29.7	67.8
Daptomycin			115[a]								100	100	100	100	100
Doxycycline					69		20	23	3[b]		84.4	72.9	89.7	51.4	77.4
Erythromycin				78[a]					37[b]		80.0	60.0	85.9	29.7	67.8
Linezolid			1[a]		63	50	1				100	100	100	100	100
Nitrofurantoin									114	1	100	100	100	100	100
Oxacillin			77[a]		3[c]	6	2	1	2	24[b]	91.1	51.4	98.7	0	66.9
Penicillin	28	5	3	1	2		76[b]				26.6[d]	21.4[d]	50	0	23.5[d]
QDA				115[a]							100	100	100	100	100
Rifampin				115[a]							100	100	100	100	100
SXT				54[a]	25	1	4	31[b]			84.4	60.0	92.3	21.6	69.6
Tigecycline			115[a]								100	100	100	100	100
Vancomycin			14[a]		100	1					100	100	100	100	100

284

285 [a] MIC ≤ value in column header; [b] value ≥ value in column header; [c] includes 1 isolate that was *mecA* negative; [d] includes 5 human isolates
286 and 1 animal isolate that had penicillin susceptible MICs but were beta-lactamase positive

287 QDA, quinupristin-dalfopristin; SXT, trimethoprim-sulfamethoxazole

288 MIC values to left of vertical lines fall in the susceptible interpretive category; those to the right are in the intermediate or resistant

289 category