Paper Ring instructions

Version 1.0 / 2020

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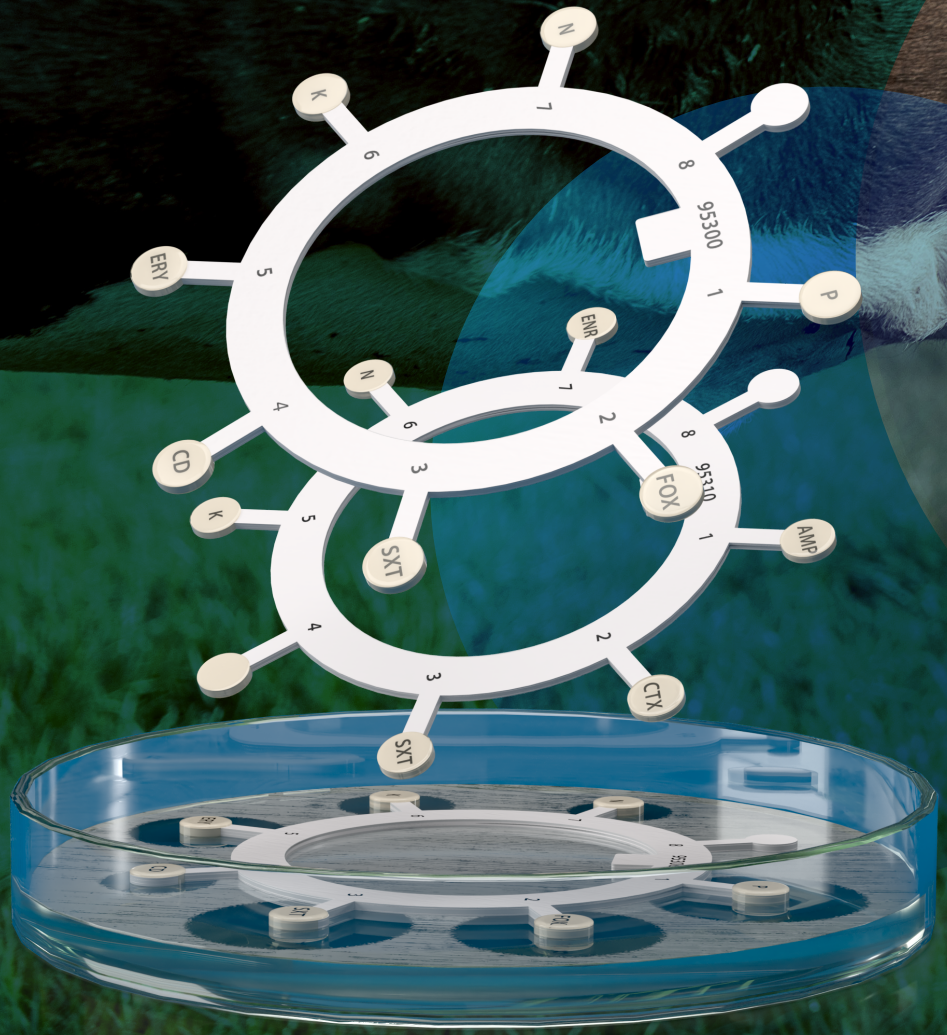
MASTITIS



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How to use?

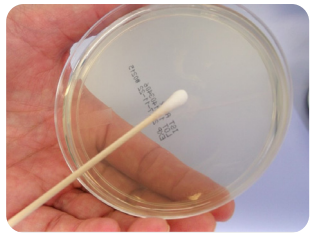
1. Allow rings to reach room temperature before opening the container (+/- 5 min).
This is to prevent condensation, leading to rapid deterioration of some agents.

2. Prepare a suspension of a fresh, pure culture. Suspend 3-5 pure colonies of an overnight culture on non-selective agar in sterile saline (0.85% solution of NaCl in water (8.5 g/L).

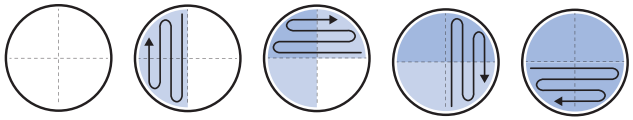
3. Mix on a vortex mixer. Adjust the turbidity equivalent to that of a 0.5 McFarland standard.

4. Dip a sterile cotton swab in the 0.5 McFarland.

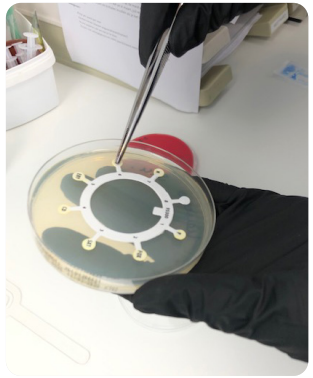
5. Spread the adjusted suspension over the dried surface of a suitable plate medium, e.g. Mueller Hinton agar.



Spread evenly over the entire surface of the agar, by streaking the swab in a standard pattern.



6. Apply the ring firmly to the inoculated agar plate within 15 minutes following the inoculation of the agar, by using tweezers ensuring complete contact with the agar surface.



7. Invert and incubate the plates at 35 ± 1°C in air for 16-20 hour.

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How to store?

100 rings in a plastic container. 10 rings are packed in a transparent plastic bag with a silica gel desiccant capsule.

Unopened bag packages: Store at –20°C to +8°C in the container provided till the expiry date; storage at –20°C is preferable if products are not used for long periods.

Opened bag packages: Leftover rings from an opened bag package must be stored at 2-8°C in the bag containing desiccant and in the plastic container provided for no more than 14 days.
Allow rings to equilibrate to room temperature before opening the container for minimizing condensation on the outer surface.

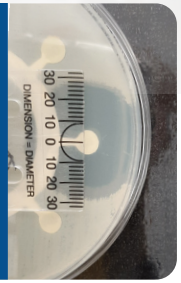
Return unused rings to the refrigerator as soon as the application of the rings has been completed. Dispose of expired rings.

How to read results?

1. Check that growth is confluent and evenly distributed over the agar surface.

   Repeat the test if not.

2. Measure inhibition zones for routine isolates. Check with quality control strains if inhibition zones are within acceptable ranges is recommended.



3. Interpret zone diameters into susceptibility categories according to the bacteria specific breakpoints:
   - S = susceptible
   - I = intermediate
   - R = resistant

The route of administration should be taken into account alongside the EMA categorisation when prescribing antibiotics.

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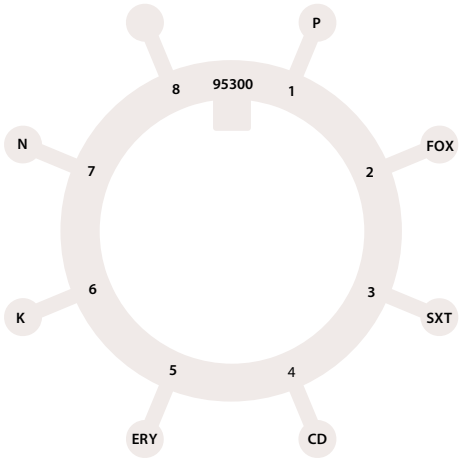
Gram positive mastitis panel



	Antibiotic	Bacterial species and diameter zones		Antibiotic class & represents	EMA category
P	Penicillin	*S. aureus*	R S; <26 ≥26	Penicillins *Represents penicillin G / benzylpenicillin / amoxicillin / ampicillin*	D Prudence
		coag. neg. *Staph.* (NAS)	R S; <26 ≥26		
		Streptococcus spp.	R S; <18 ≥18		
FOX	Cefoxitin Used for MRSA screening	*S. aureus* (MRSA)	R S; <22 ≥22	Cephalosporins	B Restrict
		coag. neg. *Staph.* (NAS)	R S; <25 ≥25		
SXT	Trimethoprim – Sulfamethoxazole	*Staphylococcus* spp.	R I S; <14 ≥17	Trimethoprim-sulphonamides *Represents all sulfa-combinations*	D Prudence
		Streptococcus spp.	R I S; <15 ≥18		
CD	Clindamycin Test in combination with erythromycin for inducible lincosamide resistance (D-test)	*Staphylococcus* spp.	R I S; <19 ≥22	Lincosamides *Represents pirlimycin and lincomycin*	C Caution
		Streptococcus spp.	R S; <17 ≥17		
Ery	Erythromycin	*Staphylococcus* spp.	R I S; <18 ≥21	Macrolides *Represents tylosin (full cross resistance with tylosin)*	C Caution
		Streptococcus spp.	R I S; <18 ≥21		
K	Kanamycin	*Staphylococcus* spp.	R I S; <14 ≥18	Aminoglycosides	C Caution
N	Neomycin	*Staphylococcus* spp.	R S; <14 ≥14	Aminoglycosides	C Caution

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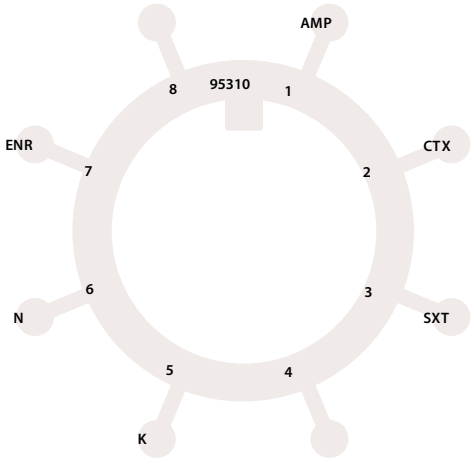
Gram negative mastitis panel



	Antibiotic	Bacterial species and diameter zones		Antibiotic class & represents	EMA category
AMP	Ampicillin	Enterobacterales	R S <14 ≥14	Penicillins	D Prudence
CTX	Cefotaxime ESBL / AmpC screening	Enterobacterales	R S <20 ≥20	Cephalosporins	B Restrict
SXT	Trimethoprim – Sulfamethoxazole	Enterobacterales	R I S <13 ≥16	Trimethoprim-sulphonamides *Represents all sulfa-combinations*	D Prudence
K	Kanamycin	Enterobacterales	R I S <14 ≥18	Aminoglycosides	C Caution
N	Neomycin	Enterobacterales	R S <12 ≥12	Aminoglycosides	C Caution
ENR	Enrofloxacin	Enterobacterales	R I S <17 ≥23	Quinolones *(e.g. Enrofloxacin)*	B Restrict

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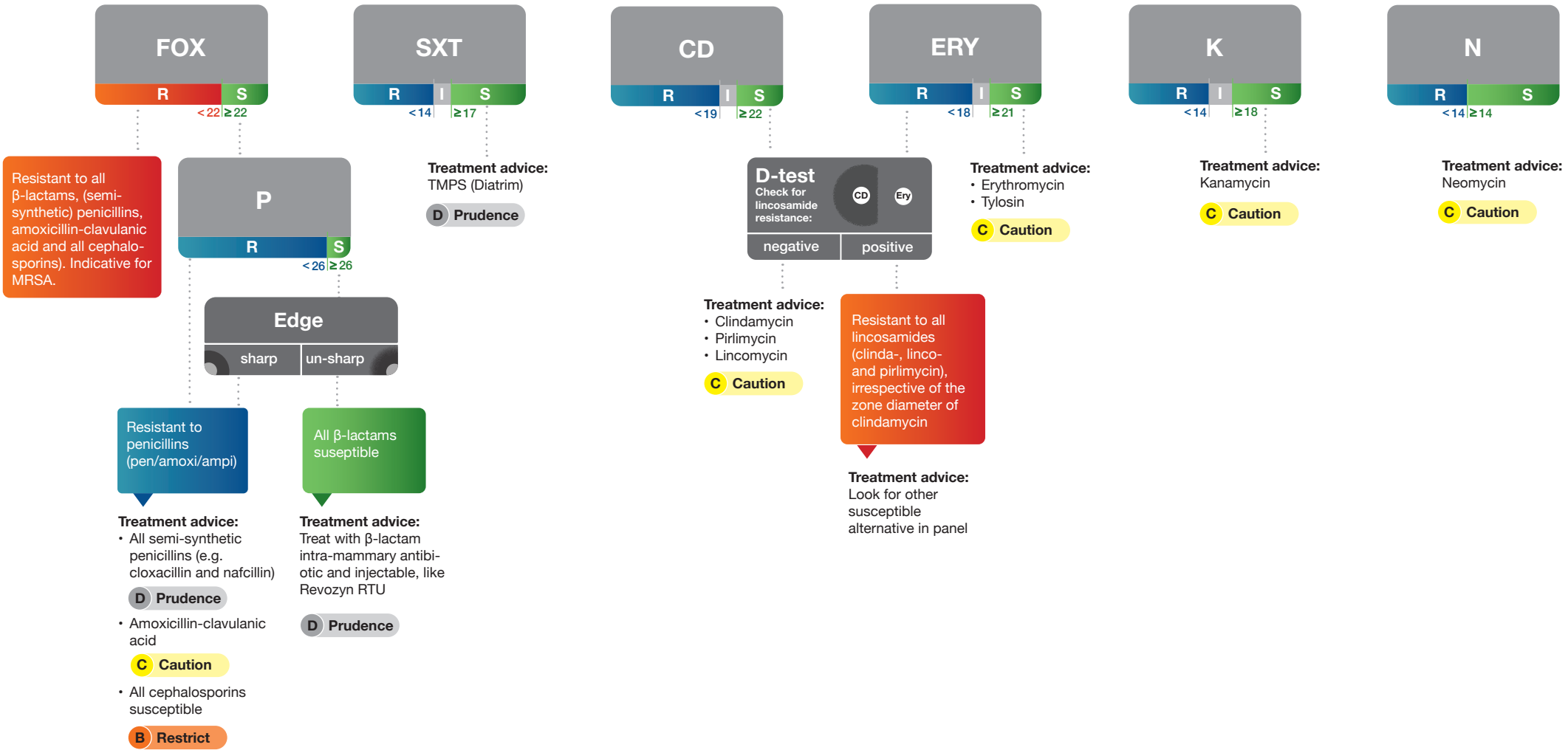
S. aureus



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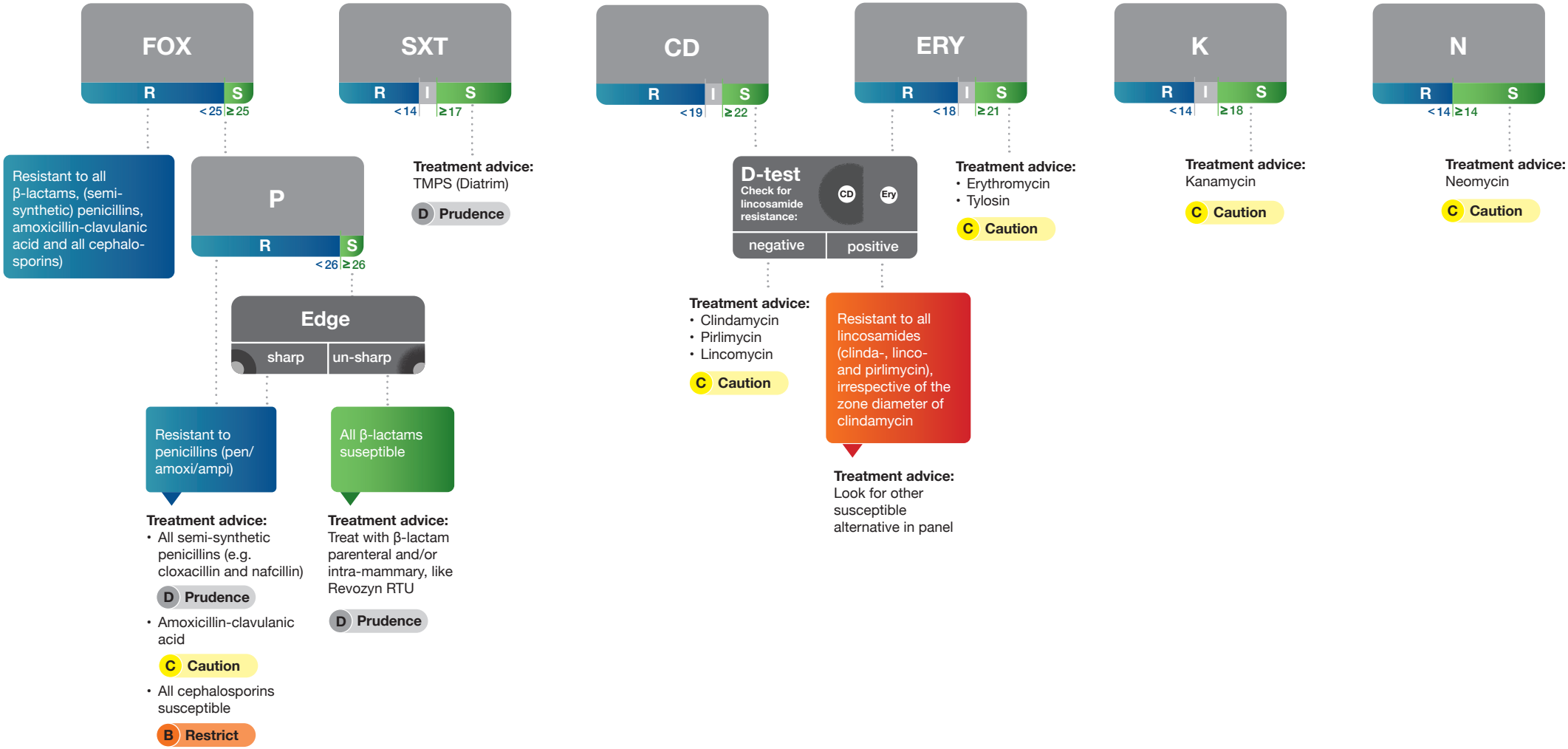
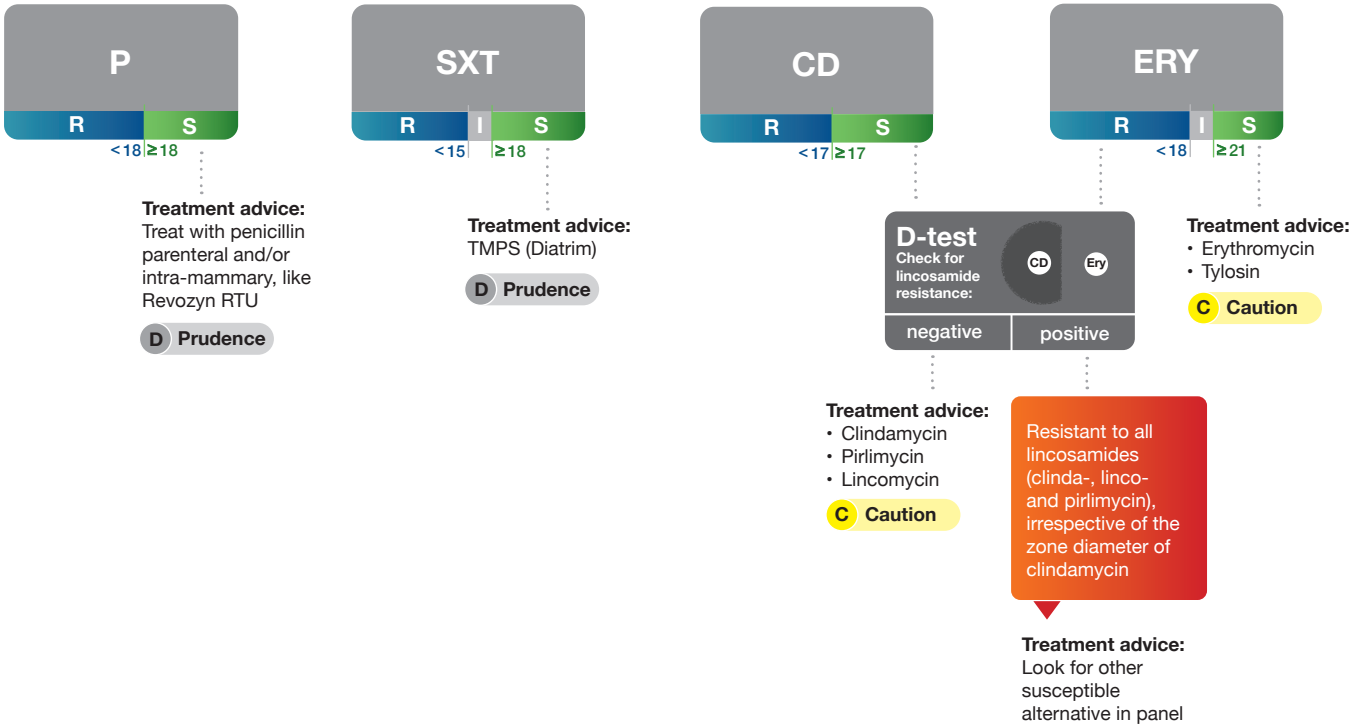
coag. neg. *Staph.* (NAS)



Streptococcus spp.

Intrinsically resistant to aminoglycosides (kanamycin, neomycin)



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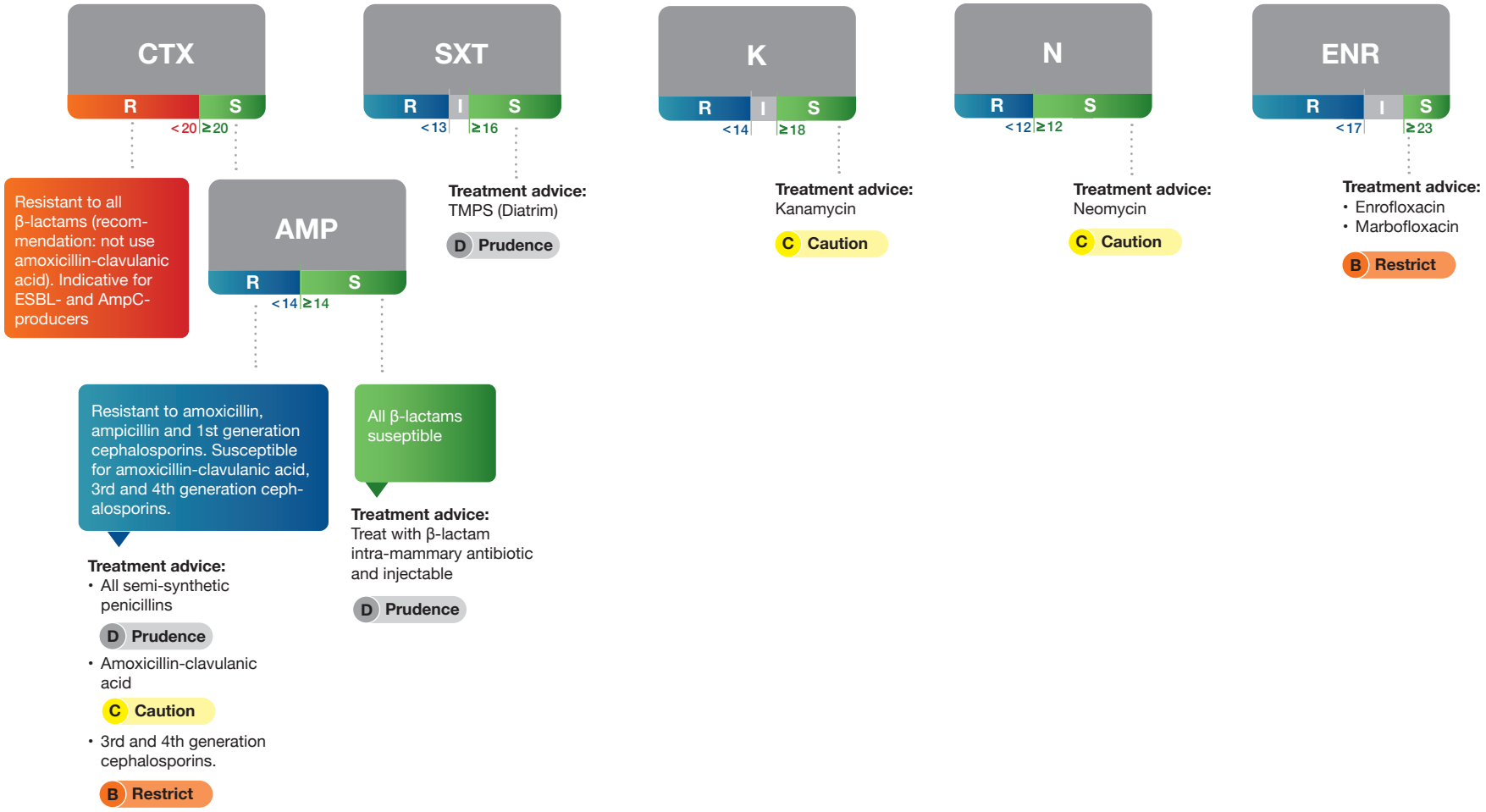
E. coli / Klebsiella spp.

E. coli is intrinsically resistant to (benzyl)penicillin. *Klebsiella* spp. are intrinsically resistant to (benzyl)penicillin and ampicillin



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