ANTIMICROBIAL RESISTANCE

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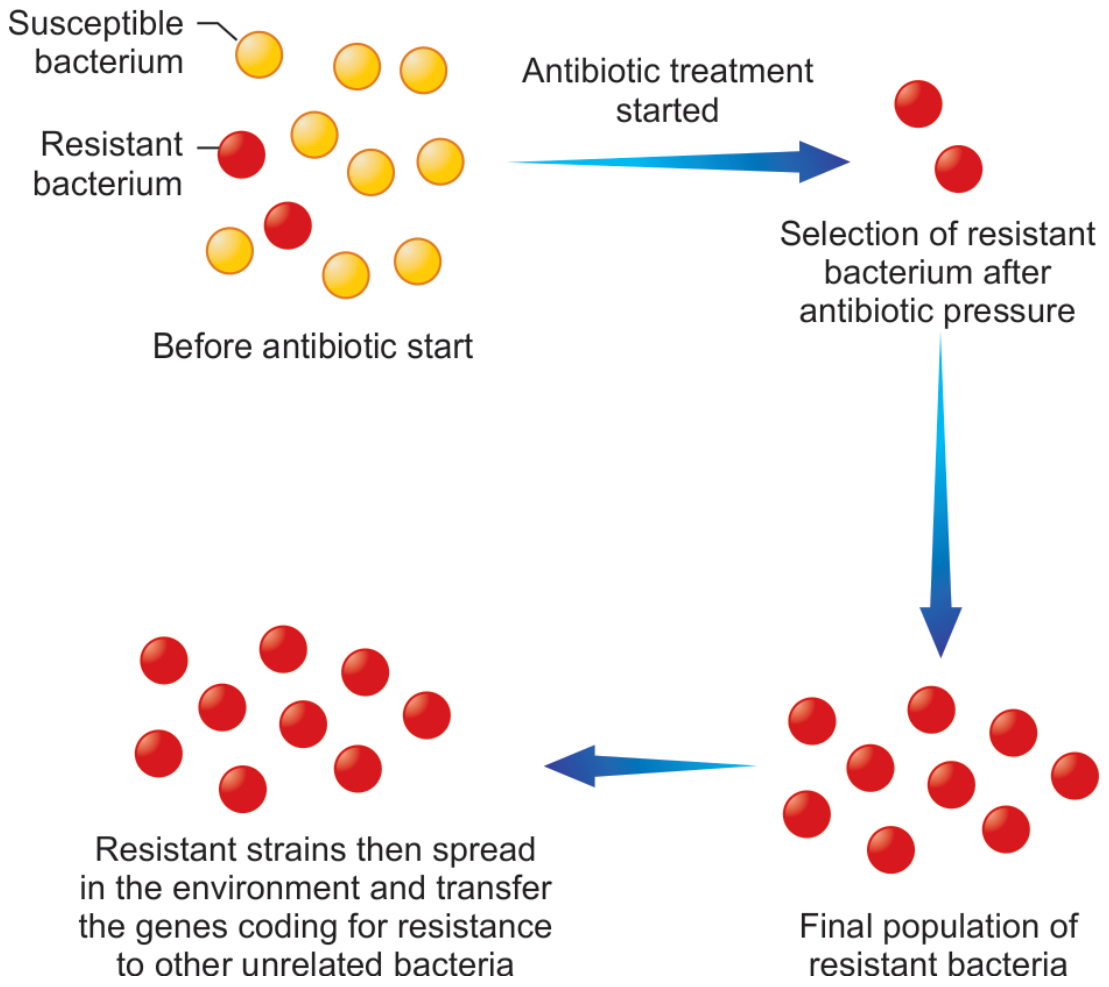
↑ Antimicrobial Use leads to AMR



AMR Status

Trend of AMR in India in humans (as per WHO country office)	2010	2020
Methicillin resistant *Staphylococcus aureus* (MRSA)		
Carbapenem resistant isolates of *Escherichia coli*		
Carbapenem resistant isolates of *Klebsiella pneumoniae*		
In *Salmonella* Typhi isolates, resistance to fluoroquinolones		
Shigella, resistance to nalidixic acid, norfloxacin, and ampicillin		
Pseudomonas species resistance to piperacillin-tazobactam		
Pseudomonas species resistance to meropenem		
Acinetobacter species resistance to meropenem		
Klebsiella pneumoniae resistance to colistin		

AMR in Gram-negatives

Resistance to Beta lactams

- β-lactamase enzymes
- Loss of porin channels
- Efflux pumps
- Alerted protein binding protein

Gram-positives

β-lactamase enzymes

- Extended-spectrum β-lactamase (ESBL)
- AmpC β-lactamase
- Carbapenemases

ESBL

- Hydrolyze: penicillins, cephalosporins (1st to 4th gen.), and monobactams
- Overcome by the addition of a β-lactamase inhibitor (BLI)
  - Clavulanate
  - Sulbactam, tazobactam, avibactam, vaborbactam etc.

ESBL detection

- **CLSI ESBL Screening test**
  - Indicators—cefotaxime, ceftazidime, c…am
  - Method: kb-DD or BMD (MIC) method
  - Screening cut-off (zone≤, MIC≥): diffe
  - Screen positive →confirmatory test
- **CLSI ESBL confirmatory test**
- Cefotaxime and cefotaxime-clavulanate
- Ceftazidime and ceftazidime-clavulanate

Zone ≥5mm
MIC ratio ≥8

ESBL testing is not clinically indicated

Interpretation using clinical BP		ESBL confirmatory test	Mechanism of resistance
BL	BLBLI		
R	S	Positive	
R	R	Positive	
S	S	Positive	
R	S	Negative	
R	R	Negative	
S	S	Negative	
S	R	Regardless	Not possible

AmpC β-lactamase detectio

- Hydrolyze: penicillins, cephems (1st, 2nd
  cephamycins), and monobactams.
- Not-inhibited by classical BLIs, especially c
- Do not hydrolyze 4th gen cephems (e.g. cefepime)

Resistant to older BLBLI with penicillins/cephems :

-Amoxicillin-clavulanate
-Ticarcillin-clavulanate
-Ampicillin-sulbactam
-Cefoperazone-sulbactam
-Piperacillin-tazobactam

AmpC types

(1) Chromosomal (natural):

- Constitutive hyperproduction (e.g. *E. coli,*
- Inducible de-repression

(2)Plasmid-mediated (acquired)

- Mainly constitutively (e.g. *E. coli, Shigella* spp
  Salmonella enterica, Citrobacter koseri, and *P. mirabilis*) or
- Rarely inducible

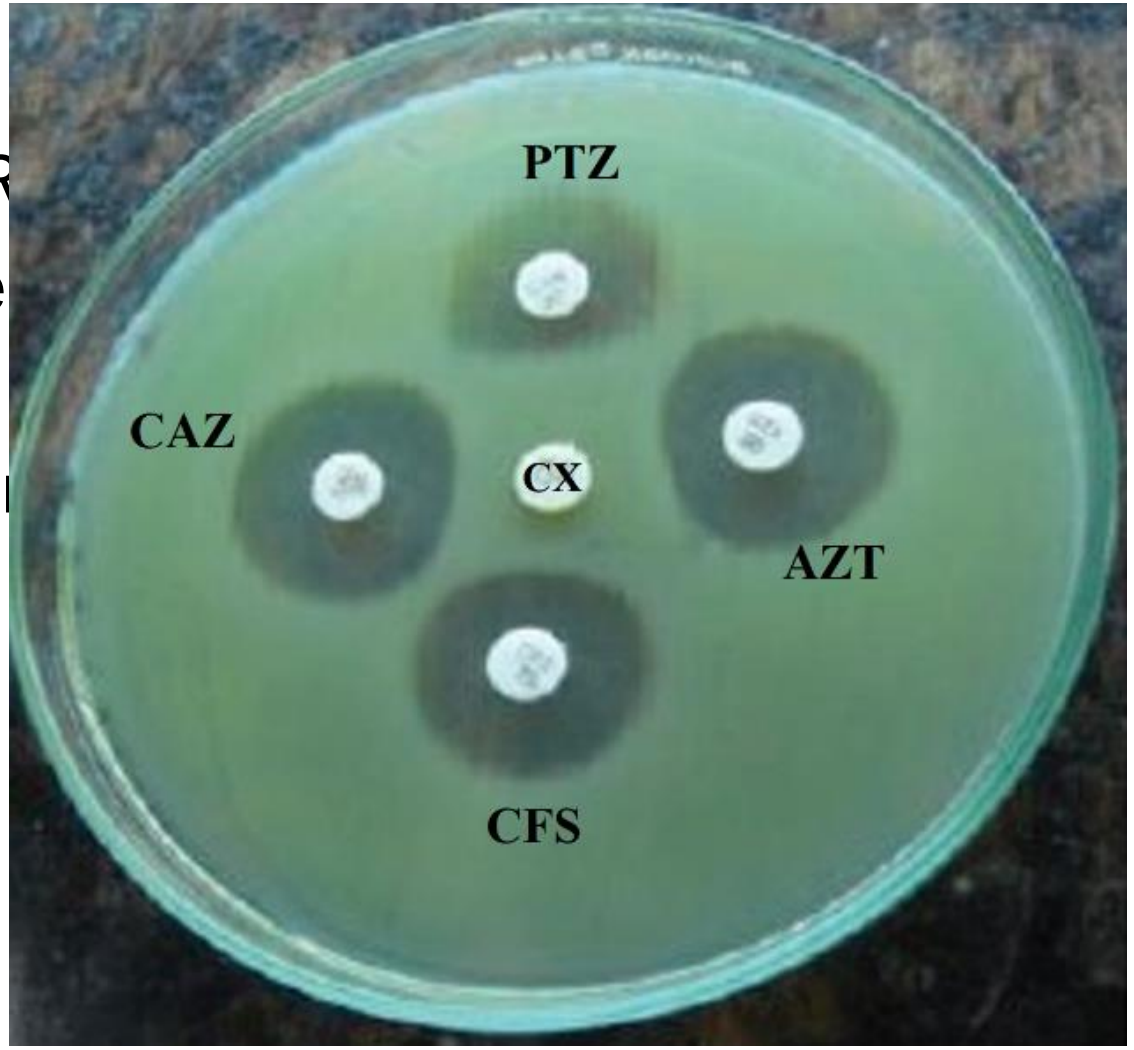
SPICE organisms

***S**erratia*
***P**seudomonas*
Indole-positive *Proteeae (Morganella, Providencia),*
***C**itrobacter freundii* complex, and
***E**nterobacter cloacae, E. aerogenes* (*K. aerogenes*)
Others: *Hafnia alvei* group, *Aeromonas group,* and *Acinetobacter baumannii* group

AmpC β-lactamase detection

AmpC Screening test:

- Cefoxitin screen test (ECOFF) + R
- S to cefepime + R to ceftazidime
- Disk Antagonism test
  - Inducer: Cefoxitin, carbapene
  - Substrate: 3rd gen cephems,



AmpC β-lactamase detection cont..

AmpC confirmation tests

- Boronic acid and Cloxacillin Combination DD test
- Boronic acid and Cloxacillin Double disk synergy test
- Three-dimensional extract test

AmpC β-lactamase detection cont..

AmpC confirmation tests

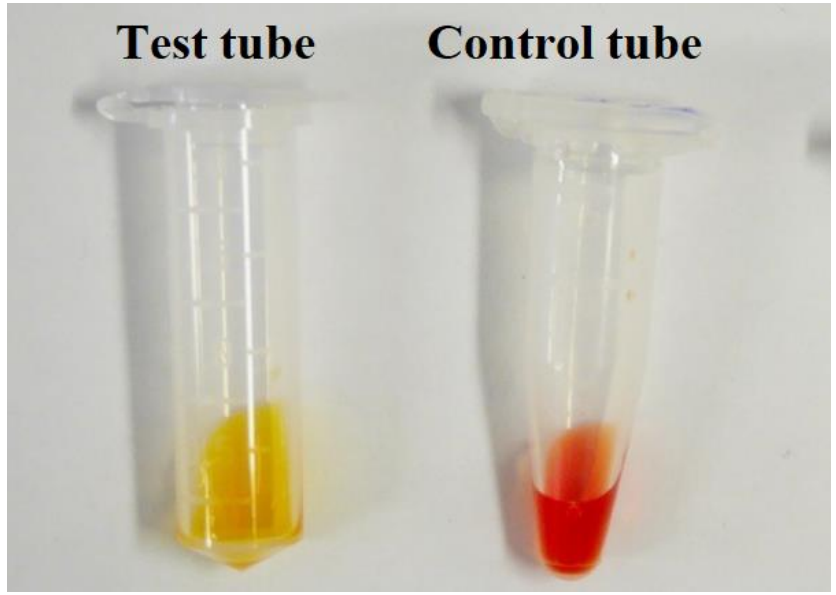
Combination DD test	Double disk synergy test (20mm gap)
Cefoxitin 30µg disk	Cefoxitin 30µg
Cefoxitin+phenylboronic acid disk	Phenylboronic acid disk
Cefoxitin+cloxacillin disk	Cloxacillin disk
Positive test is indicated by: ≥5mm -Cefoxitin- boronic acid disk -Cefoxitin - cloxacillin disk When compared to the alone	Positive test is indicated by: -Augmentation of cefoxitin zone -Keyhole pattern in Cx zone towards BA/clox. disk -Phantom zone between Cx and BA or clox. disks

Carbapenemase Detection

Carbapenemase	Genes (KOVIN)	Ambler class	Bush Jacoby group	Detection (CLSI)	Other method
Serine carbapenemases	KPC	A	2f	CarbaNP mCIM ± eCIM	BioFire Carba R ICT
	Oxa-48 type	D	2df		
Metallo β-lactamase	VIM IMP NDM	B	3		

CarbaNP Test (CLSI)

- Test strain+ imipenem + protein extraction reagent + indicator
- Carbapenemase ⟶ hydrolyze imipenem ⟶acid ⟶ phenol red ⟶ change the color
- Rapid turn-around time (<2h)
- Detects all types, except Oxa-48



Carbapenem Inactivation Methods

- mCIM: Test strain + Meropenem⟶kb-DD (ATCC *E. coli*)
  - Carbapenemase ⟶hydrolyze meropenem ⟶R/ATCC *E. coli*
- eCIM : Test strain + EDTA + Meropenem ⟶kb-DD (ATCC *E. coli*)
- EDTA ⟶ destroy MBL, retain serine carbapenemases

mCIM	eCIM	Interpretation
Positive	Positive	Metallo-β-lactamase
Positive	Negative*	Serine carbapenemase +ve
Negative	Do not interpret	Carbapenemase -ve
Indeterminate		Perform another test

Multiplex lateral flow immunoassay (NG-test CARBA5)

- C, control band
- K, band for KPC
- O, band for
- Oxa-48-like
- V, band for VIM
- I, band for IMP
- N, band for NDM band

Direct molecular test (from blood)

- BioFire FilmArray: 24 targets from blood (TAT 1h)
  - 5 carbapenemases: KPC, Oxa-48-like, VIM, IMP, NDM
  - MecA/C, Van A/B genes, CTX-M,
- Carba R: Detects 5 carbapenemase genes (TAT 2h)

Synergy testing for Aztreonam and Ceftazidime-avibactam

- Aztreonam: Stable to MBLs
- MBL producers: Co-produce ESBL/AmpC
- ESB/AmpC: Destroy aztreonam
- Avibactam: inhibit ESBL/AmpC
- Aztreonam + Ceftazidime-avibactam

Penicillins	Oxacillins	1st/2nd gen. cephems	3rd gen. Cephems	4th gen. Cephems	Aztreonam	BLBLI	Carbapenem	Ambler class	Bush Jacoby group	Interpretation (type of β-lactamase production)

AMR in Gram-positives

Oxacillin resistance

- MRSA and MRCoNS
- MecA and Mec C mediated resistance

AST method / *Staphylococcus* spp.	Cefoxitin MIC	Cefoxitin DD	Oxacillin MIC	Oxacillin DD	Oxacillin screen agar
S. aureus	✓	✓	✓	X	✓
S. lugdunensis	✓	✓	✓	X	X
S. epidermidis	X	✓	✓	✓	X
S. pseudintermedius	X	X	✓	✓	X
S. schleiferi	X	X	✓	✓	X
Other species or	X	✓	✓	X	X

Oxacillin MIC	Cefoxitin MIC	Confirmation	Interpretation
S	S	No need	MSSA (mecA-ve)
R	R	No need	MRSA (mecA +ve)
S	R	Cefoxitin DD Oxacillin screen agar	MRSA (mecC +ve)
R	S		BORSA

Vancomycin resistance in *S. aureus*

- VRSA
- VISA
- hVISA
- Avoid vancomycin: ≥2µg/mL

Organism	Source	S	I	R
S. aureus	CLSI	≤2	4-8	≥16
	EUCAST	≤2	-	≥4
CoNS	CLSI	≤4	8-16	≥32
	EUCAST	≤4	-	≥8

Vancomycin resistance in *Enterococcus*

- VR *E. faecium*
- VR *E. faecalis*
- VR *E. gallinarum* and *E. casseliflavus*

Annual Report

Antimicrobial Resistance Research and Surveillance Network

ICMR-AMRSN 2020

January 2020 to December 2020

Division of Epidemiology and Communicable Diseases

INDIAN COUNCIL OF MEDICAL RESEARCH
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Enterobacterales (S%): ICMR-AMRSN 2020

	Pip-taz		Cefotax		Ceftazid		Ertapen		Imipen		Meropen		Colistin		Amikacin		Ciproflox		Levoflox	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
C. freundii	129	41	102	22	97	27	91	49	131	53	124	56	29	97	132	58	120	47	89	58
C. koseri	251	65	244	42	176	40	197	70	252	67	250	74	21	95	257	74	244	67	133	50
Citrobacter spp	47	74	42	48	22	41	46	89	38	84	53	85	4	100	49	92	46	80	14	93
K. oxytoca	133	37	116	22	104	20	109	48	124	46	124	55	22	100	132	55	133	33	85	22
K. pneumoniae	8669	37	7658	19	5334	22	6255	41	8392	45	7771	47	2061	94	8828	47	7218	34	4913	28
Klebsiella spp	359	51	277	35	267	37	346	54	97	44	354	55	14	93	286	53	258	44	175	41
Enterobacter cloacae	839	63	739	40	489	37	559	75	863	72	814	75	91	97	872	78	812	67	311	66
Enterobacter spp	312	69	283	26	271	32	162	72	216	74	321	81	56	98	303	77	203	64	186	61
K. (E.) aerogenes	74	53	72	31	63	21	62	42	69	61	76	59	8	100	75	45	65	42	57	26
P. mirabilis	922	90	766	50	597	44	510	81	881	61	939	85			927	62	813	41	436	37
P. rettgeri	38	74	27	52	25	44	21	81	34	74	38	76			37	70	34	56	16	50
P. stuartii	117	56	105	30	94	34	38	76	118	56	121	67			117	45	118	38	31	29
E. coli	7890	53	6835	16	5072	19	5729	71	7191	72	7499	76	1065	99	7935	81	7092	22	3762	19
M. morganii	7890	53	6835	16	5072	19	5729	71	7191	72	7499	76			7935	81	7092	22	3762	19
S. marcescens	219	83	245	59	170	49	212	92	192	89	293	90			292	83	271	76	131	67
Overall	27889	**50**	24346	**20**	17853	**22**	20066	**62**	25789	**62**	26276	**67**	3371	**96**	28177	**69**	24519	**30**	14101	**26**

Enterobacterales- Urine (S%)
ICMR-AMRSN 2020

	E. coli		K. pneumoniae		K. oxytoca		Klebsiella spp		E. cloacae		Enterobacter spp		P. mirabilis		C. koseri		C. freundii		M. morganii		P. rettgeri		Overall	
	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S	n	%S
Pip-taz	7660	68	2655	47	74	51	40	30	165	66	49	67	270	89	172	77	47	55	72	85	38	21	11242	63
Cefazolin	2720	22	893	21																			3690	22
Cefotaxime	6664	24	2250	26	66	32	40	18	153	46	42	33	238	56	166	58	31	23	61	52	28	14	9739	26
Ertapenem	6541	78	2304	53	52	71	41	29	131	79	22	68	231	86	152	78	39	64	69	72	25	16	9607	72
Imipenem	7785	80	2708	59	77	68	42	55	168	71	48	69	256	55	178	79	46	48	73	53	34	12	11415	73
Meropenem	7279	83	2419	62	74	64	42	33	156	78	50	76	276	88	172	80	44	57	78	79	36	14	10626	77
Amikacin	8131	84	2823	59	81	78	42	50	174	79	54	72	275	72	181	83	47	64	81	79	38	11	11927	77
Ciprofloxacin	7364	29	2461	40	83	31	41	34	159	69	40	65	242	50	171	75	44	43	72	39	38	11	10715	33
Levofloxacin	3676	26	1348	33	39	46	35	29	52	65	28	57	131	41	73	53	31	55	29	38	20	5	5462	29
Cotrimoxazole	6656	43	2330	41	73	48	33	45	123	67	44	59	228	40	128	70	42	50	60	40	32	22	9749	43
Fosfomycin	3691	98																					3691	98
NFT	7935	83	2716	33	83	70	38	26	167	44	52	48	208	16	178	65	42	45	63	22	29	7	11511	68

K. pneumoniae: β-lactamase genes ICMR-AMRSN 2020

	RC-02		RC-08		RC-01		RC-04		RC-13		RC-12			Overall	
	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+	No tested	%+		No tested	%+
NDM	30	47	42	50	30	37	59	17	30	47	59	34		308	37
IMP	30	33	42	43	30	10	59	10	30	20	59	7		308	25
VIM	30	63	42	45	30	37	59	54	30	77	59	27		308	47
KPC	30	17	42	12	30	7	59	54	30	10	59	2		308	18
TEM	30	60	42	29	30	43	59	53	30	7	59	46		308	41
SHV	30	50	42	36	30	40	59	42	30	17	59	44		308	42
OXA-1	30	23	42	40	30	47	59	20	30	40	59	44		308	39
CTXM-1	30	53	42	38	30	30	59	27	30	57	59	56		308	45
CTXM-2	30	0	42	0	30	3	59	2	30	3	59	2		308	1
CTXM-9	30	3	42	2	30	3	59	2	30	0	59	2		308	2
CTXM-8/25	30	0	42	0	30	0	59	0	30	0	59	0		308	0
OXA-48	30	53	42	38	30	63	59	24	30	63	59	56		308	48

Pseudomonas aeruginosa ICMR-AMRSN

AMA	Year-2016 Total n=1056 (S%)	Year-2017 Total n=5687 (S%)	Year-2018 Total n=8880 (S%)	Year-2019 Total n=12634 (S%)	Year-2020 Total n=7839 (S%)
Piperacillin-tazobactam	705/1036 (68.1)	3757/5450 (68.9)	6034/8499 (71)	8416/11430 (73.6)	5012/7418 (67.6)
Cefepime	585/981 (59.6)	3074/5003 (61.4)	5259/8284 (63.5)	7660/12038 (63.6)	4497/7355 (61.1)
Ceftazidime	624/1035 (60.3)	3602/5504 (65.4)	5663/8598 (65.9)	7545/11977 (63)	4647/7635 (60.9)
Imipenem	809/1016 (79.6)	4059/5514 (73.6)	5627/8377 (67.2)	6425/10230 (62.8)	4411/7036 (62.7)
Meropenem	650/969 (67.1)	3490/5083 (68.7)	5736/8292 (69.2)	8255/12242 (67.4)	4955/7661 (64.7)
Colistin*	711/723 (98.3)	1727/1738 (99.4)	983/1075 (91.4)	1767/1899 (93)	1291/1355 (95.3)
Amikacin	693/1030 (67.3)	3864/5609 (68.9)	6019/8747 (68.8)	8340/12329 (67.6)	5276/7723 (68.3)
Gentamicin	402/776 (51.8)	2526/4249 (59.4)	4077/6462 (63.1)	5820/9383 (62)	3241/5341 (60.7)
Tobramycin	579/957 (60.5)	2954/4365 (67.7)	3809/5603 (68)	4627/6783 (68.2)	2907/4331 (67.1)
Ciprofloxacin	436/842 (51.8)	2930/5069 (57.8)	4814/8026 (60)	6281/10945 (57.4)	3768/6541 (57.6)
Levofloxacin	536/958 (55.9)	3236/5351 (60.5)	4794/8217 (58.3)	6148/10922 (56.3)	3771/6743 (55.9)

Acinetobacter baumannii : ICMR-AMRSN

AMA	Year -2016 Total=396	Year -2017 Total=3359	Year -2018 Total=4549	Year -2019 Total=8531	Year -2020 Total=6849
	(S%)	(S%)	(S%)	(S%)	(S%)
Piperacillin-tazobactam	94/335 (28.1)	484/3187 (15.2)	760/4494 (16.9)	1245/8010 (15.5)	770/6724 (11.5)
Cefepime	67/318 (21.1)	368/3300 (11.2)	587/4457 (13.2)	1040/8271 (12.6)	587/6571 (8.9)
Ceftazidime	56/328 (17.1)	355/3202 (11.1)	575/4164 (13.8)	905/7453 (12.1)	546/6441 (8.5)
Imipenem	104/334 (31.1)	501/3346 (15)	818/4517 (18.1)	1098/7272 (15.1)	744/6702 (11.1)
Meropenem	100/331 (30.2)	615/3287 (18.7)	953/4178 (22.8)	1742/8399 (20.7)	779/6747 (11.5)
Colistin*	*0/0	28/31 (90.3)	36/38 (94.7)	103/108 (95.4)	91/94 (96.8)
Amikacin	102/347 (29.4)	638/3312 (19.3)	877/3795 (23.1)	1429/7016 (20.4)	1014/5863 (17.3)
Minocycline	*0/0	926/1380 (67.1)	2393/3725 (64.2)	3893/6431 (60.5)	2794/5139 (54.4)
Levofloxacin	104/312 (33.3)	886/3040 (29.1)	959/4047 (23.7)	1500/7841 (19.1)	825/6181 (13.3)

S. aureus ICMR-AMRSN 2020

AMA	All Specimens			
	S. aureus n=6280	MSSA n=3655	MRSA n=2582	CoNS n=2018
Cefoxitin	3394/5787 (58.6%)	3388/3388 (100%)	0/2399 (0)	487/1907 (25.5%)
Oxacillin	1140/1869 (61%)	1100/1100 (100%)	40/769 (5.2%)	*4/4 (-)
Penicillin	251/3608 (7%)	231/1931 (12%)	0/1652 (0)	134/1391 (9.6%)
Vancomycin	3846/3846 (100%)	2153/2153 (100%)	1676/1676 (100%)	890/890 (100%)
Teicoplanin	2043/2050 (99.7%)	1074/1075 (99.9%)	948/953 (99.5%)	229/238 (96.2%)
Erythromycin	2594/6096 (42.6%)	1962/3570 (55%)	621/2490 (24.9%)	396/1999 (19.8%)
Tetracycline	4734/5284 (89.6%)	2838/3047 (93.1%)	1885/2223 (84.8%)	1582/1916 (82.6%)
Tigecycline	1559/1559 (100%)	861/861 (100%)	694/694 (100%)	117/117 (100%)

AMA	Year-2016	Year-2017	Year-2018	Year-2019	Year-2020
	Total n=960	Total n=5708	Total n=8644	Total n=12320	Total n=6281
	(S%)	(S%)	(S%)	(S%)	
Cefoxitin	686/958 (71.6)	3805/5668 (67.1)	4863/7919 (61.4)	6272/10835 (57.9)	3394/5787 (58.6)
Oxacillin	*0/0	314/438 (71.7)	1218/2196 (55.5)	2280/3773 (60.4)	1140/1869 (61)

Enterococcus ICMR-AMRSN 2020

AMA	All Specimens (except urine)	
	***Enterococcus faecalis* n=1189**	***Enterococcus faecium* n=1206**
Ampicillin	945/1124 (84.1)	123/1071 (11.5)
Vancomycin	1137/1170 (97.2)	916/1185 (77.3)
Teicoplanin	1146/1170 (97.9)	952/1188 (80.1)
Gentamicin HL	621/1084 (57.3)	345/994 (34.7)
Linezolid	1152/1169 (98.5)	1136/1198 (94.8)

AMA	Urine	
	***Enterococcus faecalis* n=912**	***Enterococcus faecium* n=788**
Ampicillin	661/818 (80.8)	77/739 (10.4)
Vancomycin	881/903 (97.6)	630/781 (80.7)
Teicoplanin	855/869 (98.4)	618/759 (81.4)
Gentamicin HL	438/734 (59.7)	232/702 (33)
Ciprofloxacin	127/585 (21.7)	38/544 (7)
Nitrofurantoin	811/894 (90.7)	319/779 (40.9)
Fosfomycin	482/497 (97)	Not tested
Linezolid	722/728 (99.2)	677/698 (97)

Thank you