



Microbiology Newsletter

Sir Ganga Ram Hospital

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Diagnosis of TB at Sir Ganga Ram Hospital

Should Gold Standard for TB diagnosis be redefined?

Smear Examination

Out of the various techniques known for AFB smear examination, Ziehl-Neelsen (ZN) has been used most extensively. But Direct Fluorescence (DF), using fluorescent stains such as Auramine-Rhodamine, is a superior alternative to ZN stain. Because of increased sensitivity and shorter time required for screening, fluorochrome stains, recommended by the CDC (Centers for Disease Control and Prevention), are preferred.¹ In our experience, this technique has paid rich dividend in detecting 1+ (low positive) acid fast bacilli from the ultrasound, CT or MR guided aspirates and tissue samples, where the yield of organisms is dismally low (Fig 1). The overall positivity of DF in culture positive samples has been 77.8% as compared to 55.1% for ZN staining. In case of pulmonary samples also, DF has shown a greater sensitivity as compared to ZN staining. As a result of the above findings, we prefer fluorescence stain -DF, to the routine ZN staining in detecting AFB in our hospital setting. Prasanthi and Kumari² also found fluorescence staining to be more efficient (45%) when compared to ZN staining (29%) in detecting pulmonary tuberculosis associated with HIV seropositivity, especially in paucibacillary cases.

Culture and Identification

Conventional culture of mycobacteria on solid media is labour intensive and time consuming. Substantial improvement in time to detection and total number of positive cultures can be achieved if automated or semi-automated liquid culture systems such as MB-BacT/ALERT-3D (MBBA-3D) (bioMérieux) and BACTEC™ 460TB/9000MB (BD) that use Middlebrook broth are used. In our experience, we have found the nonradiometric MBBA-3D system to be a rapid and sensitive method to isolate mycobacterial species from both pulmonary and extra-pulmonary clinical specimens. 875 samples were cultured on both the MBBA-3D system and Lowenstein-Jensen (LJ) medium, out of which 88 were positive in either or both systems. MBBA-3D and LJ medium recovered 94.3% and 76.1% mycobacterial isolates, respectively. It appears that MBBA-3D system is more sensitive than conventional LJ. Also, the MBBA-3D system detected mycobacteria more rapidly than the LJ medium (15.2 versus 26.5 days for smear positive specimens and 24.6 versus 40.1 days for smear-negative specimens). Smear positive samples with higher mycobacterial load, i.e. 3-4+ positive are usually positive within 7-10 days.

Identification of mycobacteria by growth characteristics and conventional biochemical tests is time-consuming, often requiring 3 to 6 weeks or even longer after sufficient growth is present on solid media. Rapid identification of isolates of mycobacterial species can be done using AccuProbe (GenProbe, USA) system. It includes tests for the identification of MTB complex, *M. avium* complex, *M. avium*, *M. intracellulare*, *M. kansasii* and *M. gordonae* is available. In our experience, the system appears to be promising. It is highly specific and identifies mycobacterial species within 2 hours. Out of the 92 mycobacterial isolates identified by AccuProbe, 83 belonged to MTB complex, 2 to *M. avium* complex and 5 still remained unidentified even after using all the five probes mentioned above. One of these was identified as *M. chelonae* by conventional techniques and rest 4 remained unidentified.

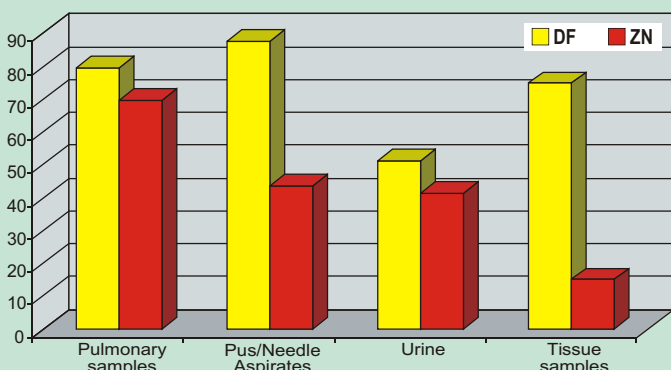
Molecular Assays (Direct Amplification Tests - DAT)

The most recent technological advance towards the rapid diagnosis of mycobacterial disease that has taken the fancy of most clinicians has been the development of molecular tools for amplifying specific DNA or RNA sequences directly from the clinical material. Commercially available standardised nucleic acid based amplification techniques have been shown to yield reliable results within 5-7 hours of sample processing, but these assays have to be used intelligently. All these nucleic acid amplification techniques have shown good sensitivity and specificity for respiratory tract specimens. In contrast, there is considerably less experience with direct detection of MTB in extra-pulmonary specimens. Paradoxically, it is the extrapulmonary situation of TB, for which a rapid and accurate laboratory diagnosis is often sought for, since the traditional techniques of detecting AFB have failed to deliver due to the small amount of bacteria normally present in these specimens, thus emphasising greater need for these techniques for nonrespiratory specimens.

In our experience, of the 136 samples (both pulmonary and extra-pulmonary) tested simultaneously for AFB smear, culture and NASBA (Nucleic acid based amplification assay) the overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of NASBA compared with the AFB (DF) results has been 70, 85, 68 and 86 percent, respectively. Smear positive and NASBA negative results could be due to prior antitubercular treatment or NTMs (Nontuberculous mycobacteria). Smear negative and NASBA positive results can be explained by the fact that the nucleic acid amplification tests are more sensitive than AFB smear results. The overall sensitivity, specificity, PPV and NPV of NASBA compared with AFB culture have been 75, 75, 34 and 95 percent, respectively. The sensitivity and specificity of NASBA as compared to culture was higher for pulmonary specimens, as compared to extrapulmonary samples, i.e. 100 and 88 as compared to 72 and 71 percent, respectively.

The dismally low PPV as compared to culture is due to the poor culture positivity, which in turn could have been due to poor specimen quality or inadequate quantity. Also culture is less sensitive in detecting non-cultivable or non-viable mycobacteria or those from paucibacillary specimens (of the 29 NASBA positive and culture negative cases, 26 were extra pulmonary cases, where culture yield is generally low). Hence nucleic acid amplification results should ideally be compared, in addition to culture, with clinical evidence of disease as known from history, radiological and other diagnostic findings. Moreover, even though NASBA/TMA (Transcription Mediated Amplification) assays are

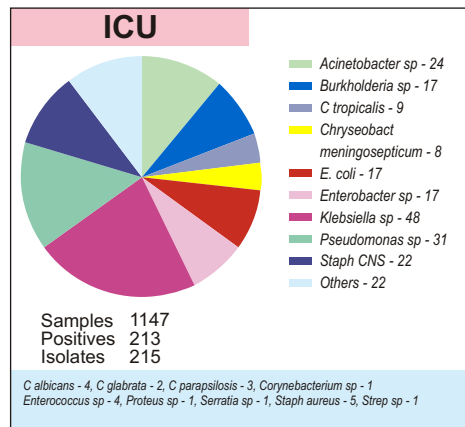
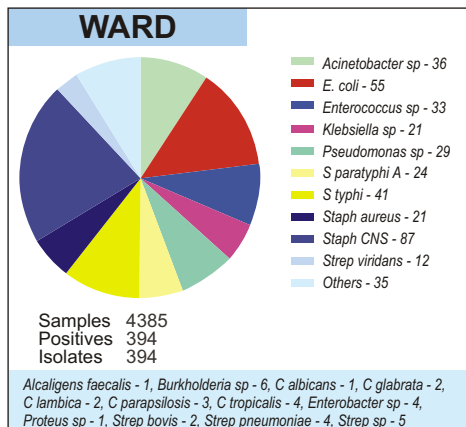
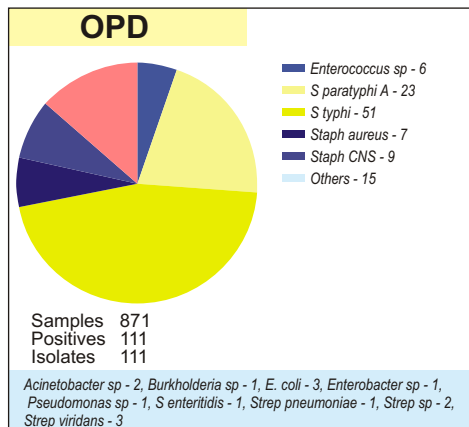
Fig. 1: Comparative Evaluation of DF and ZN Staining at SGRH
Percentage Positivity of DF and ZN Staining



contd. on page 8

BLOOD

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PERCENTAGE RESISTANCE

GPC

	No. of Isolates	Penicillin	Oxacillin**	Ampicillin	Clindamycin	Gentamicin	HLAR* Gentamicin	Teicoplanin
Staph aureus	7	86	40	-	0	60	-	0
	21	73	29	-	7	31	-	0
	5	100	40	-	0	25	-	0
Staph CNS	9	78	25	-	0	29	-	0
	87	82	67	-	39	64	-	0
	22	85	75	-	45	68	-	0
Enterococcus sp	6	-	-	0	-	-	20	0
	33	-	-	54	-	-	69	12***
	4	-	-	-	-	-	-	-
Strept. viridans	3	-	-	-	-	-	-	-
	12	8	-	-	-	-	-	0
	0	-	-	-	-	-	-	-

OPD
WARD
ICU

* HLAR: High Level Aminoglycoside Resistance.
** Oxacillin sensitivity can be extrapolated for all -lactams and -lactam-inhibitor combinations; and Teicoplanin sensitivity for Vancomycin.
*** Four isolates of VRE.

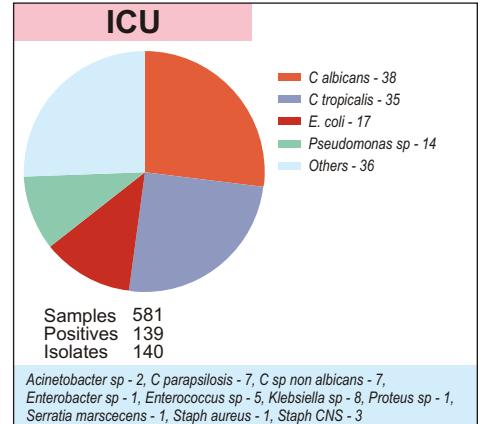
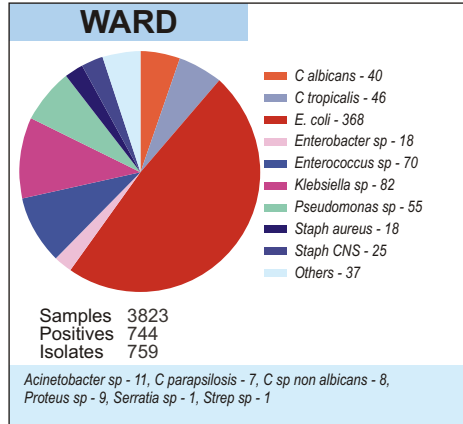
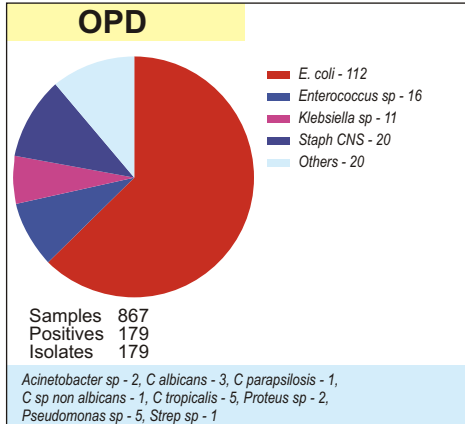
GNB

	No. of Isolates	Ampicillin	Cefuroxime	Ceftriaxone	Cefoperazone*	Ceftazidime*	Gentamicin	Nalidixic acid	Amikacin*	Ciprofloxacin*	Co-trimoxazole	Chloramphenicol	Piperacillin+ Tazobactam*	Cefoperazone+ Sulbactam*	Meropenem*
S. typhi	51	12	-	0	-	-	-	86	-	0	18	14	-	-	-
	41	20	-	0	-	-	-	86	-	3**	24	20	-	-	-
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. paratyphi A	23	0	-	0	-	-	-	95	-	4**	0	0	-	-	-
	24	0	-	0	-	-	-	95	-	5**	0	0	-	-	-
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	55	94	81	79	-	-	63	-	7	90	-	-	48	-	0
	17	100	67	67	-	-	70	-	20	67	-	-	67	-	0
Klebsiella sp	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	21	100	71	71	-	-	73	-	88	67	-	-	74	-	0
	48	100	93	93	-	-	88	-	36	81	-	-	63	-	0
Pseudomonas sp	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	29	-	-	-	84	68	84	-	74	75	-	-	51	60	55
	31	-	-	-	89	67	86	-	86	67	-	-	26	59	52
Acinetobacter sp	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	29	89	68	62	-	-	67	-	73	50	-	-	23	25	50
	20	100	92	68	-	-	85	-	84	58	-	-	43	-	48

*Percentage Resistance may indirectly appear higher than actual, because 2nd line drugs are tested only in multi-drug resistant isolates.
** Moderately susceptible (Kirby Bauer disc diffusion); resistant to Nalidixic acid; Refer to highlights on Pg. 8 for ACCO data for Salmonella.

URINE

January - June 2005



PERCENTAGE RESISTANCE

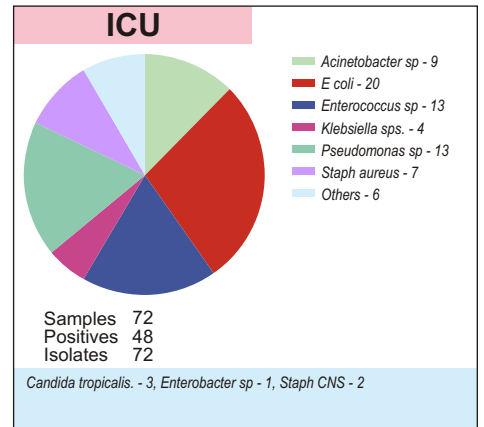
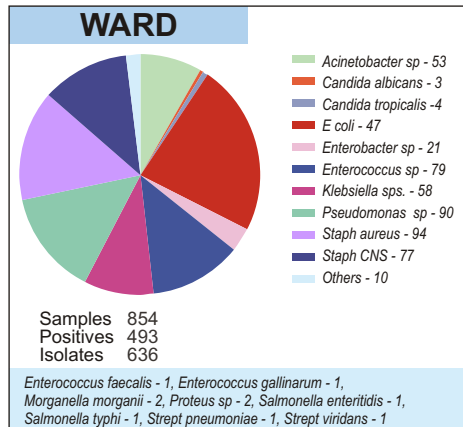
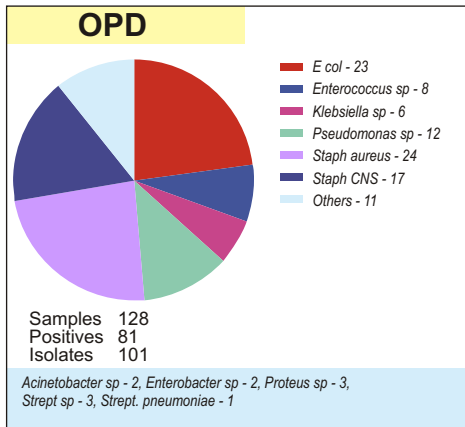
GPC	No. of Isolates	OPD								
		Penicillin	Ampicillin	Oxacillin*	Clindamycin	Nitrofurantoin	Gentamicin	HLAR** Gentamicin	Norfloxacin	Teicoplanin
<i>Enterococcus sp</i>	16	-	13	-	-	-	-	50	93	0
	70	-	55	-	-	-	-	67	97	3***
	5	-	60	-	-	-	-	100	100	0
<i>Staph CNS</i>	20	80	-	50	17	0	59	-	65	0
	25	78	-	50	0	5	74	-	95	0
	3	-	-	-	-	-	-	-	-	-
<i>Staph aureus</i>	3	-	-	-	-	-	-	-	-	-
	18	61	-	33	44	9	41	-	85	0
	1	-	-	-	-	-	-	-	-	-

OPD
WARD
ICU

* Oxacillin sensitivity can be extrapolated for all -lactams and -lactam-inhibitor combinations; and Teicoplanin sensitivity for Vancomycin.
** HLAR: High Level Aminoglycoside Resistance.
*** Two isolates of VRE.

GNB	No. of Isolates	ICU																	
		Ampicillin	Cefuroxime	Nalidixic acid	Cefotaxime	Ceftazidime*	Cefoperazone	Nitrofurantoin	Gentamicin	Netilmicin	Amikacin*	Norfloxacin	Ciprofloxacin*	Ofloxacin	Co-trimoxazole	Colistin*	Piperacillin+ Tazobactam*	Cefoperazone+ Sulbactam*	Meropenem*
<i>E. coli</i>	112	75	31	88	43	-	-	19	40	27	20	75	76	77	67	-	14	27	0
	368	91	75	93	67	-	-	28	55	41	26	86	86	85	80	-	25	40	0
	17	100	100	100	88	-	-	38	94	60	53	94	91	92	88	-	27	58	0
<i>Pseudomonas sp</i>	5	-	-	-	-	40	40	-	60	67	40	-	60	-	0	0	0	20	0
	55	-	-	-	45	58	-	72	68	63	-	66	-	-	6	34	52	42	
	14	-	-	-	75	78	-	92	86	92	-	83	-	-	0	64	73	83	
<i>Klebsiella sp</i>	11	100	50	82	64	0	-	55	78	33	18	82	78	75	80	-	27	33	0
	82	100	75	86	67	-	-	58	62	53	36	79	72	74	78	-	36	35	0
	8	100	-	100	89	-	-	75	88	60	63	100	100	83	75	-	29	50	0
<i>Enterobacter sp</i>	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	18	100	50	76	50	-	-	76	41	38	33	72	69	69	82	-	27	17	0
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Percentage Resistance may indirectly appear higher than actual, because 2nd line drugs are tested only in multi-drug resistant isolates.



PERCENTAGE RESISTANCE

GPC	No. of Isolates	Antibiotic							
		Penicillin	Oxacillin*	Gentamicin	HLAR Gentamicin**	Clindamycin	Erythromycin	Ciprofloxacin	Teicoplanin
<i>Staph aureus</i>	24	95	14	32	-	13	67	44	0
	94	87	30	40	-	21	33	47	0
	7	100	43	57	-	43	100	100	0
<i>Staph CNS</i>	17	65	53	37	-	19	-	100	0
	77	83	58	56	-	37	75	37	0
	2	-	-	-	-	-	-	-	-
<i>Enterococcus sp</i>	8	50	-	-	50	-	-	75	0
	79	38	-	67	44	-	-	75	1***
	13	100	-	-	82	-	-	100	15***

OPD
WARD
ICU

* Oxacillin sensitivity can be extrapolated for all -lactams and -lactam-inhibitor combinations; and Teicoplanin sensitivity for Vancomycin.

** HLAR: High Level Aminoglycoside Resistance.

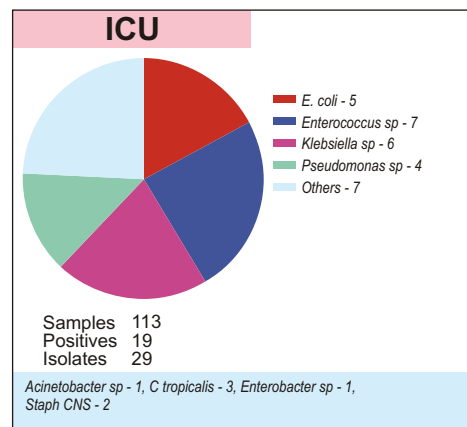
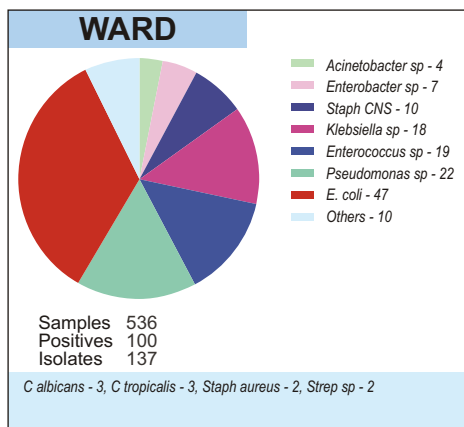
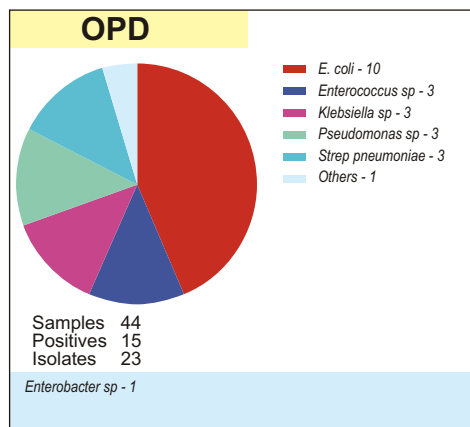
*** One and two isolates of VRE in wards and ICU, respectively.

GNB	No. of Isolates	Antibiotic													
		Ampicillin	Co-trimoxazole	Co-amoxycylav	Cefotaxime	Cefotaxime	Ceftazidime	Gentamicin	Netilmicin	Amikacin	Ciprofloxacin	Ofloxacin	Cefoperazone + Sulbactam*	Piperacillin + Tazobactam*	Meropenem*
<i>E. coli</i>	23	83	75	89	88	75	88	-	47	13	78	82	47	13	0
	147	93	80	83	68	77	68	-	53	25	84	85	42	27	0
	20	90	80	67	100	89	100	-	50	25	85	89	67	28	0
<i>Pseudomonas sp</i>	12	-	-	-	-	25	-	50	40	58	40	-	30	17	47
	90	-	-	-	-	40	-	41	48	47	52	-	47	28	40
	13	-	-	-	-	100	-	92	100	93	92	100	91	80	86
<i>Klebsiella sp</i>	6	100	20	75	100	80	100	-	67	50	0	60	100	33	0
	58	98	75	100	71	78	71	-	42	41	75	74	34	26	0
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acinetobacter sp</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	53	78	64	69	86	69	86	-	73	33	67	68	58	25	-
	9	100	100	75	100	100	100	-	100	0	100	100	67	67	100
<i>Enterobacter sp</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	21	95	71	100	50	68	50	-	20	35	71	74	75	19	0
	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus sp</i>	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	26	67	64	65	67	32	67	-	40	50	50	58	0	0	0
	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*Percentage Resistance may indirectly appear higher than actual, because 2nd line drugs are tested only in multi-drug resistant isolates.

BODY FLUIDS

January - June 2005



PERCENTAGE RESISTANCE

GPC	No. of Isolates	Penicillin	Oxacillin**	Clindamycin	Gentamicin	HLAR* Gentamicin	Teicoplanin	OPD
								WARD
<i>Enterococcus sp</i>	3	-	-	-	-	-	-	
	19	100	-	-	-	33	0	
	7	100	-	-	-	100	28***	
<i>Staph CNS</i>	-	-	-	-	-	-	-	
	10	90	50	0	44	-	0	
	2	-	-	-	-	-	-	

* HLAR: High Level Aminoglycoside Resistance.

** Oxacillin sensitivity can be extrapolated for all -lactams and -lactam-inhibitor combinations; and Teicoplanin sensitivity for Vancomycin.

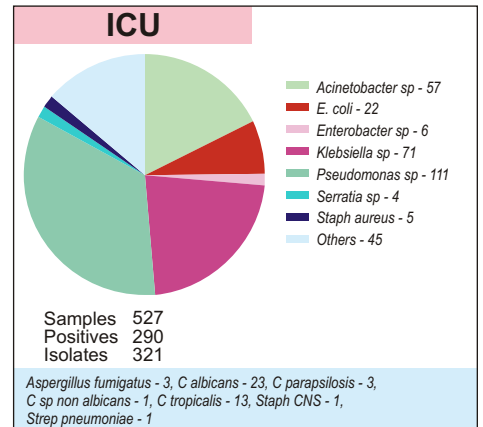
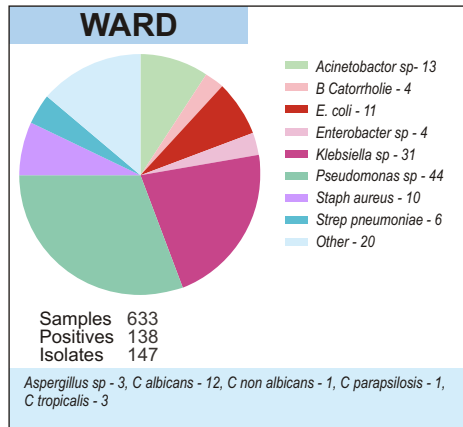
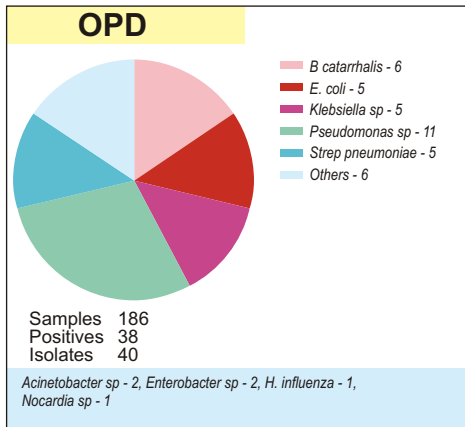
*** Two isolates of VRE.

GNB	No. of Isolates	Ampicillin	Cefuroxime	Ceftriaxome	Piperacillin	Gentamicin	Netilmicin	Amikacin	Ciprofloxacin	Ofloxacin	Co-trimoxazole	Piperacillin + Tazobactam*	Cefoperazone + Sulbactam*	Meropenem*	OPD
															WARD
<i>E. coli</i>	10	82	44	100	100	44	28	20	90	100	55	16	33	0	
	47	94	82	78	90	65	42	16	90	92	78	37	52	0	
	5	100	75	100	100	100	100	40	20	100	100	-	75	0	
<i>Pseudomonas sp</i>	3	-	-	-	-	-	-	-	-	-	-	-	-	-	
	22	-	-	-	55	79	58	50	75	85	-	21	81	43	
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Klebsiella sp</i>	3	-	-	-	-	-	-	-	-	-	-	-	-	-	
	18	100	100	78	100	70	60	40	70	66	80	31	30	0	
	6	100	83	-	50	83	60	16	50	50	83	100	66	0	

*Percentage Resistance may indirectly appear higher than actual, because 2nd line drugs are tested only in multi-drug resistant isolates.

RESPIRATORY ISOLATES

January - June 2005



PERCENTAGE RESISTANCE

GPC	No. of Isolates	Penicillin	Oxacillin*	Clindamycin	Erythromycin	Gentamicin	Ciprofloxacin	Tetracycline	Vancomycin*
Staph. aureus	-	-	-	-	-	-	-	-	-
	10	70	10	0	33	0	60	33	0
Strept. pneumoniae	5	0	0	0	0	-	0	0	0
	6	16	16	0	0	-	16	0	0
	1	-	0	-	-	-	-	-	-

* Oxacillin sensitivity can be extrapolated for all -lactams and -lactam-inhibitor combinations; and Vancomycin sensitivity for Teicoplanin.

GNB	No. of Isolates	Ampicillin	Ceftazidime	Ciprofloxacin	Co-trimoxazole	Gentamicin	Amikacin	Netilmicin	Cefoperazone + Sulbactam*	Piperacillin + Tazobactam*	Meropenem*
Pseudomonas sp	11	10	20	33	-	36	28	17	13	22	18
	44	-	48	61	-	71	61	87	52	38	43
	111	-	70	82	-	89	87	88	76	64	81
E. coli	5	100	-	75	40	40	40	33	100	25	0
	11	100	-	91	100	60	37	25	25	22	0
	22	100	-	100	91	91	32	50	67	80	0
Klebsiella sp	5	100	-	20	20	20	16	33	-	33	0
	31	100	0	74	63	55	51	50	50	40	0
	71	100	100	88	90	87	49	65	50	45	2
Acinetobacter sp	2	-	-	-	-	-	-	-	-	-	-
	13	100	66	72	60	60	79	57	15	16	69
	57	97	80	93	75	76	92	56	35	57	78

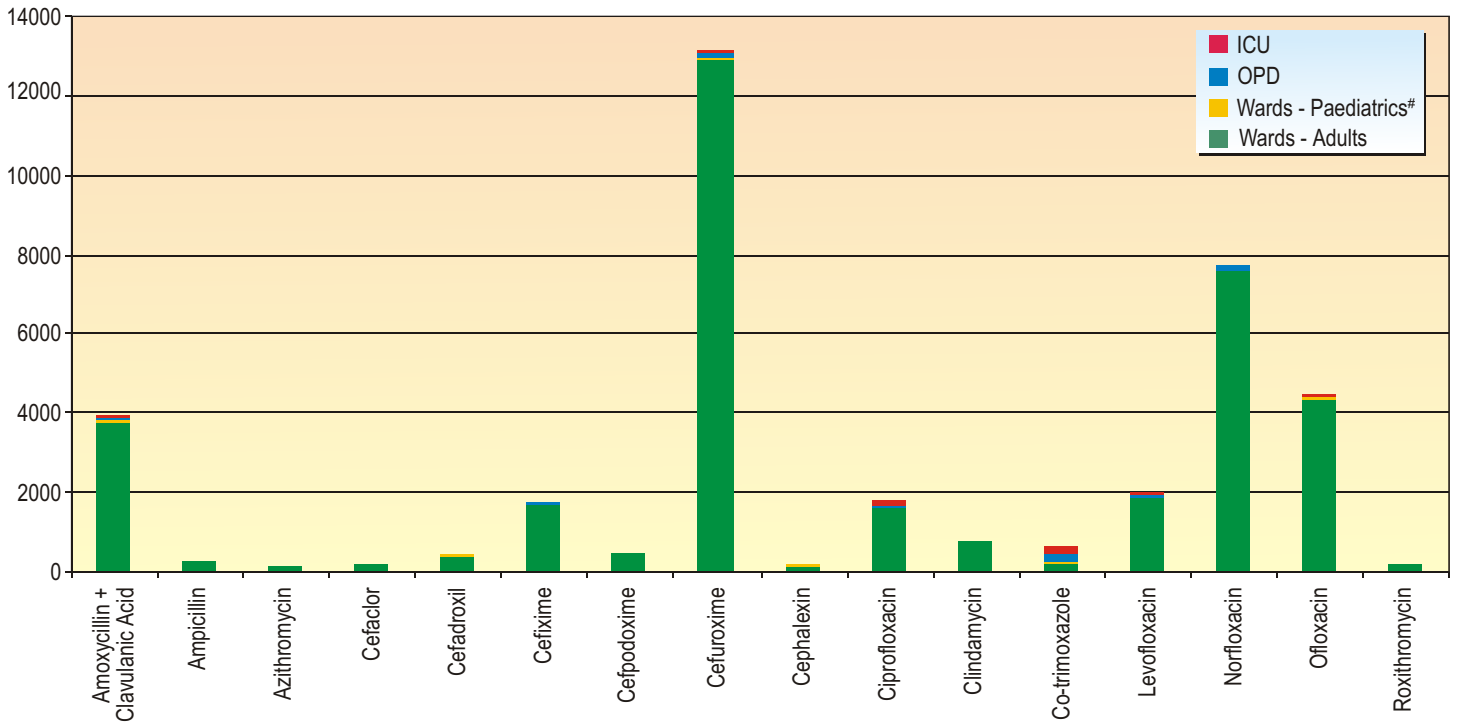
*Percentage Resistance may indirectly appear higher than actual, because 2nd line drugs are tested only in multi-drug resistant isolates.

PRESCRIPTION AUDITING

January - June 2005*

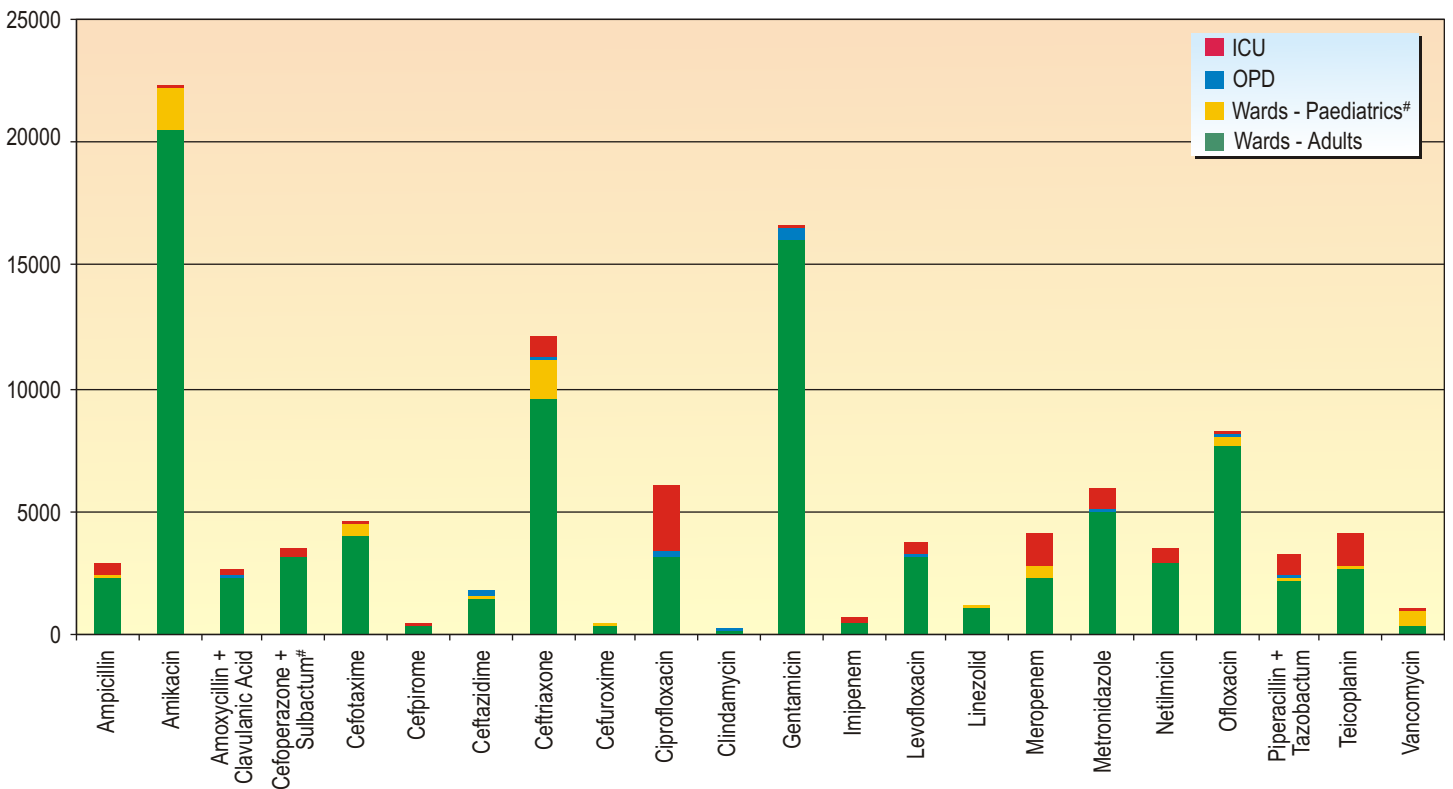
ORAL

DDD**



PARENTERAL

DDD**



* Based on the hospital pharmacy data of the antibiotics dispensed.

** DDD - Daily Defined Doses; calculated as per the Anatomical Therapeutic Chemical (ATC) classification index, WHO Collaborating Centre for Drug Statistics Methodology, Oslo, Norway.

In paediatric group of patients and for certain antibiotics, consumption calculated as per the "Guide to antimicrobial therapy" by Sanford et al, USA (2005) presuming 10 kg as the average body weight for paediatric patients.

primarily recommended (FDA) for pulmonary (smear positive and smear negative) cases, in our study we mostly had extrapulmonary samples and patients on therapy for investigation. It is critical for all TB molecular assays to exclude cases that are on ATT or who have received other antibiotics (like quinolones, aminoglycosides, etc) having anti-mycobacterial activity.

False negative NASBA results (NASBA negative, culture positive) were most likely due to sampling errors (sample inhomogeneity), the presence of possible amplification inhibitors, which have been reported to be present in as high as 20% of samples in literature^{3,4} and/or low RNA levels due to poor viability of mycobacteria (due to whatever reasons, especially in patients on antitubercular therapy). In our experience, we have found that 9% of samples processed for NASBA had inhibitors and that inhibitors persisted in 5% samples even after using different procedures for their removal. The samples mostly having inhibitors were, urine, CSF, ascitic fluid. It is essential to know with certainty the samples detected as negative is a true negative. To ensure this, for quality control of NASBA, an internal control - U1A cell control, Universal sample control U1A - mRNA encoding U1 fraction snRNP, found in all human cells in abundance, was added to the samples after the isolation step for monitoring the amplification and detection steps. Non detection of the U1A indicated the presence of inhibitors. Hence, all the NASBA negative samples where U1A was positive were true negative samples.

Even though literature says cultures are more sensitive than smears as a technique, but due to low culture positivity rate due to varied reasons, the time has come to rethink regarding the “gold standard” in tuberculosis diagnostic tests.⁵ It appears no single assay like culture can be the gold standard, but a combination of assays with good clinical evidence can help in diagnosing tuberculosis more often than by cultures alone.

TMA based Amplified Mycobacterium Tuberculosis Direct Test (AMTDT- Gen-Probe, USA), which is an FDA approved assay, for direct detection of *Mycobacterium tuberculosis* in both smear positive and smear negative pulmonary samples is presently being standardised in our laboratory, primarily for respiratory specimens and appears to be equally promising as NASBA..

A workshop under the American Thoracic Society (1996) discussed the clinical laboratory and public health aspects of the direct amplification tests (DATs) performed on primary specimens. The main conclusion of the report was that the tests are a major improvement over standard techniques, although not enough information existed on their clinical and public health implications. Wherever direct amplification test was positive with a high clinical suspicion of TB, the implication was to treat, but in absence or where clinical suspicion of tuberculosis was low, if smear was positive but DATs negative, the clinical details were considered and repeat testing was done. Final recommendation was that DAT should always be correlated with microscopy and/ or culture and that the result should be interpreted along with the clinical data. Local test performance and underlying TB prevalence are also important variables that will have an impact on the decision for treatment/ therapy. Molecular test being expensive cannot be used on all specimens nor can any health care system recommend its routine use. We must restrict the use of highly sophisticated investigations and utilize such assays only in situations where clinical management will be assisted by molecular tests.

The guidelines are very clear in suggesting that DAT as an unrestricted/universal test cannot be recommended. Nucleic acid amplification tests can enhance diagnostic certainty, but they do not replace AFB smear or mycobacterial culture, and the later do not replace the clinical judgement.

Problems in the of diagnosis of TB

- Primarily extra pulmonary samples received for TB diagnosis.
- Clinical / treatment details not mentioned, mostly patients on ATT subjected to investigations.

Comparative evaluation of NASBA with AFB smear (DF)

Total samples (136)	Smear positive (44)	Smear negative (92)
NASBA positive (44)	30	14
NASBA negative (92)	13+1*	78

Sensitivity - 70%; Specificity - 85%; PPV - 68%; NPV - 86%
* NTM

Comparative evaluation of NASBA with culture

Total samples (136)	Culture positive (21)	Culture negative (115)
NASBA positive (44)	15	29**
NASBA negative (92)	5*** + 1*	86

Sensitivity - 75%; Specificity - 75%; PPV**** - 34%; NPV - 95%
* NTM. ** 26 extrapulmonary samples. *** Possible causes: Faulty sample collection (not in RNase / DNase free vial) & increased transport time. Patients on ATT. Inhibitors even though these have been excluded in the study.
**** Refer text for details

References

1. Woods GL. The mycobacteriology laboratory and new diagnostic techniques. Infectious Diseases Clinics of North America 2002;16(1):127-44.
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Highlights

- The ACCo resistance in *S.typhi* is around 20%, Nalidixic acid resistance in *Salmonella typhi* and *paratyphi A* is 86% and 95% respectively.
- Isolation of MRS A in pus varied between 14 - 43%.
- Cefuoxime continues to be the most commonly used oral antimicrobial, followed by norfloxacin, ofloxacin and co-amoxyclav.
- Amongst parenteral antimicrobials, amikacin continues to be most widely used, followed by gentamicin, ceftriaxone and ofloxacin.

Publications

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- Wattal C, Joshi S, Sharma A, Oberoi JK, Prasad KJ, et al “Prescription Auditing and antimicrobial resistance at a tertiary care hospital at New Delhi, India” J Hosp Infect 2005. 59/2; 156-158
- Wattal C & Datta S. “Diagnosis of MDR Tuberculosis” J Int Med Sc Acad 2005. 18: 2; 99-103.

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