

Original Research Article

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Surveillance of Nosocomial Infections in a Private Hospital with Particular Emphasis on Characterization and Drug Resistance Pattern

D. B. Phule* and A. V. Manwar

Department of Microbiology, Dnyanopasak College of Arts, Commerce, Science, and Technology, College Parbhani Maharashtra, India

*Corresponding author

ABSTRACT

Keywords

Nosocomial infection, Surveillance, Antibiotic sensitivity, Minimum inhibitory concentration

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Nosocomial Infections (NIs) surveillance plays a vital role in reduced infection rates. Numbers of hospitals are practising the same for many years, and positive effects have demonstrated. Therefore, this study also attempted to evaluate the tendency of NIs towards drug resistance and the resultant impact of surveillance on NIs rates and advisory as a precautionary measure. The use of selective nutrient media assisted in isolating nosocomial pathogens under NIs surveillance program. The Nosocomial pathogens identified by 16S rRNA and 18S rRNA gene sequencing and tested for antibiogram and MIC assay to note the antibiotic sensitivity pattern. The private hospital's different sections found to be positive for nosocomial pathogens identified as *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecium*, *P. aeruginosa* and fungi *Candida albicans*. Further based on the antibiogram, all strains found to be multidrug-resistant with an increased MIC level making them virulent strains as per NIs surveillance program carried out in hospital of Maharashtra, India. As per NIs surveillance program carried out in hospital of India, pathogens showcased heterogeneity of prevalence as per areas, and further remained multidrug-resistant even to the third-generation drug indicated the controlling action against them is prerequisite and reported accordingly to the ethical committee monitoring the study.

Introduction

The hospitals are committed to giving the best of the facility to the patient's, but they are now experiencing the growing challenge of nosocomial infection (NI). NIs can hinder the progress of the patient's recovery further can prolong hospital stay and can increase the economic burden of both patients and hospitals. The length of stay due to NIs

reported to increase by 9.1 days with ventilator-associated pneumonia (VAP), and by 9.5 days in case of central line-associated bloodstream infection (Mehta *et al.*, 2016). Besides, as per estimated cost, additional expenditure increases by 4900 Euro for per hospital-acquired bloodstream infection (Vrijjeus *et al.*, 2010). As early as in 1983, it was proven once that surgical wound surveillance could reduce surgical site

infections (Condon *et al.*, 1983), now many publications focusing on the role of surveillance systems. In one study, for prevention of NIs, ten key points were mentioned; one of them is surveillance and feedback (Zingg *et al.*, 2015). The main points involved in surveillance were to collect information about the outbreak and to give comparable data for benchmarking potential spread.

Till date, the number of countries succeeded in establishing their national or regional surveillance system, by involving network and electronic records to transfer data fast (Woeltje *et al.*, 2011; Inacio *et al.*, 2011). Take the cognizance of the NIs surveillance, the number of hospitals is implementing the same for many years, and their impact needs to be explored. Therefore, the aim of this study to evaluate the present status of NIs in a private hospital, India about drug resistance pattern showcased by them.

Materials and Methods

Site of sampling

The incidences of multidrug-resistant human pathogens are on the rise and mainly prevalent in hospital settings that demand regular surveillance. In the study one of the private hospitals of Parbhani, Maharashtra considered for registering the drug resistance in bacterial and fungal species prevalent in hospital settings.

During sampling, swab samples collected from the bed, saline stands, ventilators, doors, patient's blood and wound. The swab then transferred to the normal saline and processed for microbial isolation of selective media.

Growth of isolates on selective media

The presence of *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter*

aerogenes, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in hospital environment confirmed by inoculation on the Hichrome agar, MacConkey agar, Blood agar, Eosin methylene blue agar, Mannitol salt agar, and Cystine-lactose-electrolyte deficient medium by incubating at 37° for 24 hours. Isolates further purified and identified as per 16S rRNA gene sequencing.

16S rRNA gene sequencing

Preliminary identified nosocomial pathogens identified by 16S rRNA gene sequence information, once its homology recorded with nucleotide database available with NCBI as per protocol suggested by Rai *et al.*, (2013).

18S rRNA gene sequencing

Fungal isolate also targeted for 18S rRNA gene locus by using universal primers: Forward primer ITS1 TCCGTAGGTGA ACCTGCGG and Reverse primer ITS2 TCCTCCGCTTATTGATATGC by setting up PCR conditions as Initial denaturation at 94°C for 2 minutes, amplification for 35 cycles with set conditions as 94°C for 45 sec, 55°C for 60 sec, and 72°C for 60 sec. Lastly extension made at 72°C for 10 minutes. Amplicon then sequenced and checked for close homology via phylogeny.

Antibiotic sensitivity assay

We identified nosocomial bacterial pathogens once tested for antibiotic sensitivity against 14 antibiotics by involving standard Kirby-Bauer disc diffusion assay. Those named as ampicillin, cefoperazone, ceftriaxone, nalidixic acid, ciprofloxacin, tetracycline, erythromycin, trimethoprim, ofloxacin, vancomycin, amikacin, gentamicin, levofloxacin and chloramphenicol.

Similarly, fungal isolate also been checked for antibiotic sensitivity against variconazole,

caspofungin, fluconazole, ketoconazole, and itraconazole.

The bacterial plates incubated at 37°C while fungal plates at 28°C for 24 h and 96 h, respectively. The formed zone of inhibition noted for prevalent sensitivity as per CLSI and EUCAST standard and isolates noted for their drug sensitivity.

Minimum inhibitory concentration assay

The antibiotics representing resistance against nosocomial pathogens tested further for MIC as per the protocol of Palomino *et al.*, (2002).

Results and Discussion

Identification of nosocomial pathogens

The prevalence of nosocomial pathogens in the hospital setting recorded once the growth of *E. coli*, *K. pneumoniae*, *S. aureus*, *E. aerogenes*, *P. aeruginosa* and fungi *C. albicans* noted on the selective media tested. The colony characteristics and typical growth-based identity then confirmed by 16S rRNA and 18S rRNA gene sequence homology.

As per 16S rRNA gene sequencing isolate DP1 with 99.2% homology identified as *E. coli* (Acc. No. MN0063) (Fig. 1a).

Isolate DP02 registered 100% sequence homology with *Klebsiella pneumoniae* (Acc. No. MN00638.1) as in Fig. 1b.

Isolate DP03 recorded 99.63% homology with 16S rRNA gene of *Staphylococcus aureus* (Acc. No. CP053183.1) as in Fig. 1c.

Isolated DP04 recorded 100% sequence identity with 16S rRNA gene of *Enterococcus faecium* (Acc. No. MK990044.1) as in Fig. 1d.

Isolate DP05 with 100% sequence match recorded homology with *Pseudomonas aeruginosa* (Acc. No. MN006381.1) as noted in Fig. 1e.

As per 18S rRNA gene sequencing isolate DP06 marked 100% homology with *Candida albicans* (Acc. No. MK998696.1) and identified as *C. albicans* as in Fig. 1f.

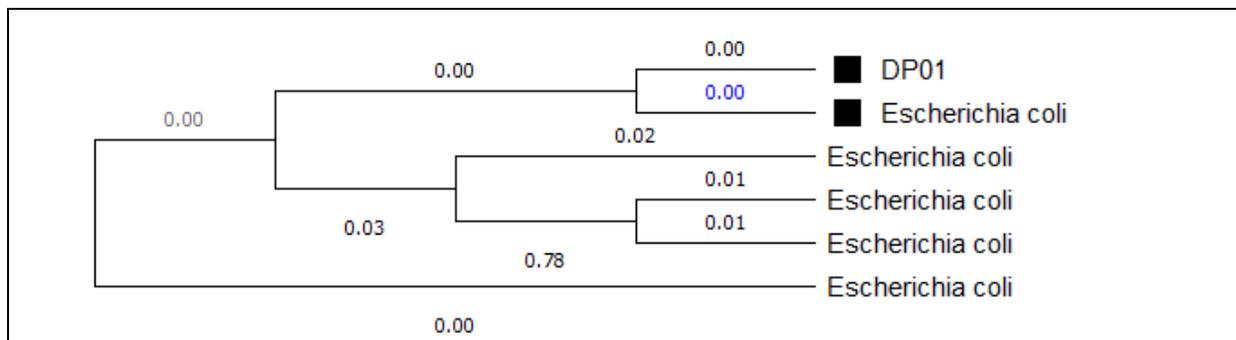


Fig.1a Nosocomial pathogen DP01 identified as *Escherichia coli* as per sequence homology of 16S rRNA gene

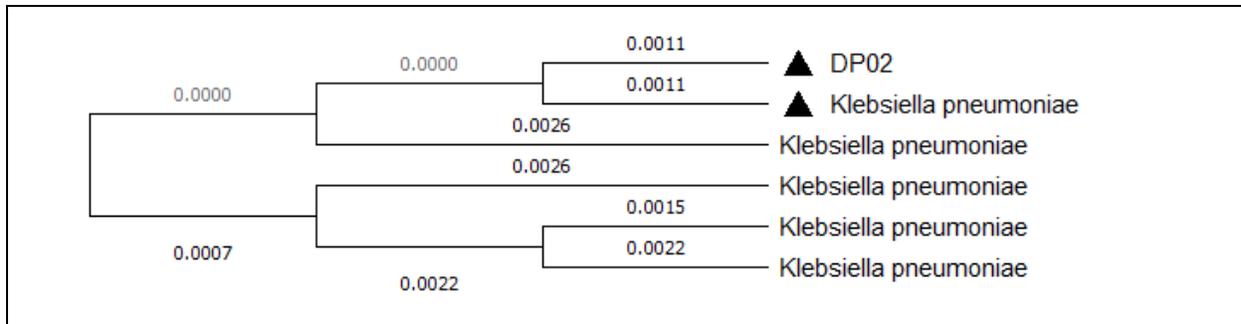


Fig.1b Nosocomial pathogen DP02 identified as *Klebsiella pneumoniae* as per sequence homology of 16S rRNA gene

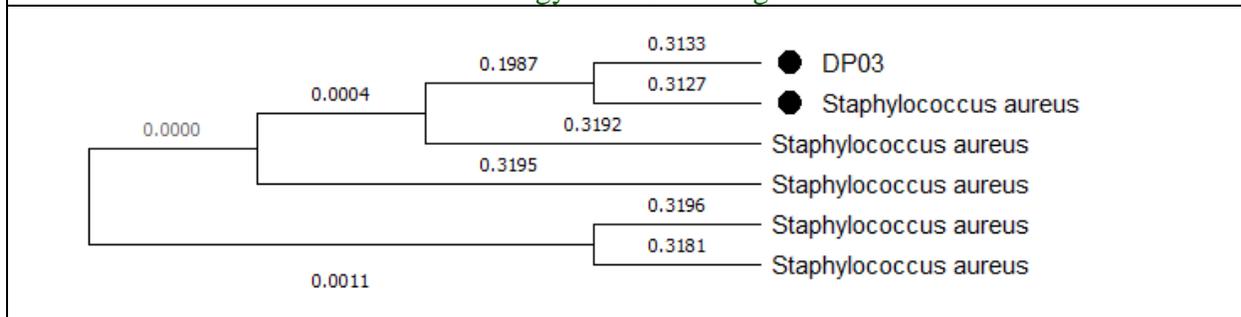


Fig.1c Nosocomial pathogen DP03 identified as *Staphylococcus aureus* as per sequence homology of 16S rRNA gene

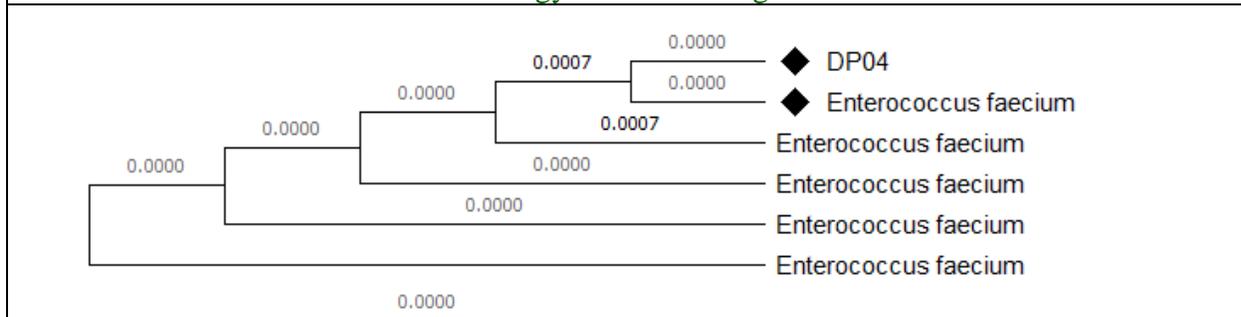


Fig.1d Nosocomial pathogen DP04 identified as *Enterococcus faecium* as per sequence homology of 16S rRNA gene

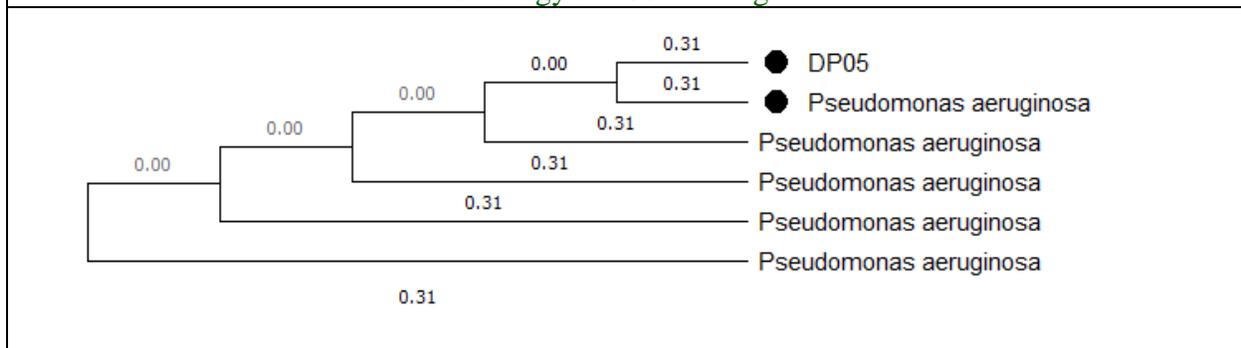


Fig.1e Nosocomial pathogen DP05 identified as *Pseudomonas aeruginosa* as per sequence homology of 16S rRNA gene

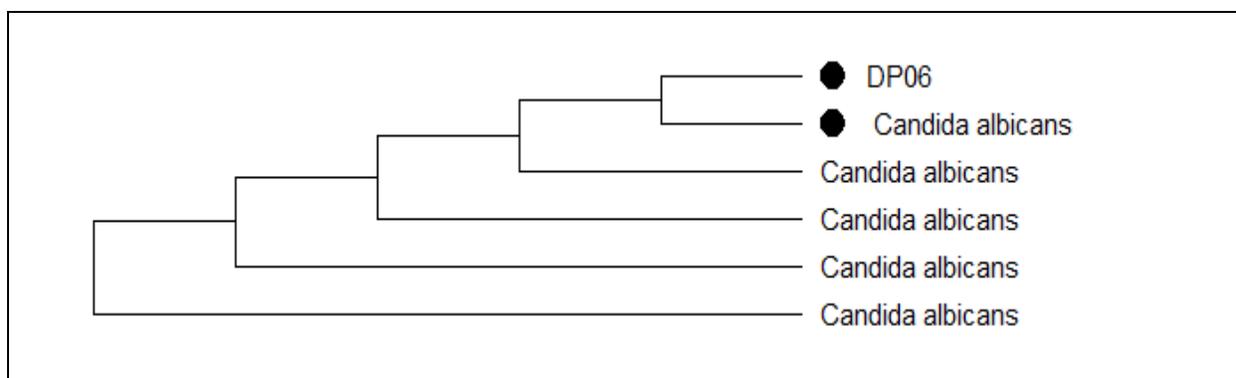


Fig.1f Nosocomial pathogen DP06 identified as *Candida albicans* as per sequence homology of 18S rRNA gene

Antibiotic sensitivity assay

Once identified by 16S rRNA and 18S rRNA gene sequencing nosocomial pathogens checked for antibiotic sensitivity as per drug class.

Ampicillin

Ampicillin sensitivity recorded with *E. coli*, *S. aureus*, *E. aerogenes* but found to be resistance with *K. pneumoniae* and *P. aeruginosa* (Table 1).

Cefoperazone

Nosocomial pathogens namely *E. coli*, *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* recorded to be resistant towards cefoperazone which is worrisome (Table 1).

Ceftriaxone

With antibiotic ceftriaxone increased resistance recorded with *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* (Table 1).

Nalidixic acid

Three nosocomial pathogens found to be resistance towards nalidixic acid namely *K. pneumoniae*, *P. aeruginosa* and *S. Aureus*

(Table 1).

Ciprofloxacin

The only resistance recorded with *P. aeruginosa* for the drug ciprofloxacin (Table 1).

Tetracycline

Tetracycline resistance noted with *K. pneumoniae* and *E. aerogenes* only (Table 1).

Erythromycin

As per CLSI standard, erythromycin registered resistance in *E. coli*, *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* (Table 1).

Trimethoprim

The most resistance against trimethoprim noted with *P. aeruginosa*, *K. pneumoniae* and *S. aureus* (Table 1).

Ofloxacin

The antibiotic resistance towards ofloxacin noted with *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* (Table 1).

Vancomycin

As a worrisome factor even, vancomycin recorded resistance with four nosocomial pathogens namely *E. coli*, *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* (Table 1).

Amikacin

In a relief point of view, all five nosocomial pathogens remained sensitive towards amikacin (Table 1).

Gentamicin

The commonly used gentamicin also noted with resistance by *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa* and others as sensitive (Table 1).

Levofloxacin

The only resistance recorded with *P. aeruginosa* and others as sensitive (Table 1).

Chloramphenicol

The isolates namely *K. pneumonia* and *E. aeruginosa* noted to be resistance towards chloramphenicol (Table 1).

The overall study reported the major drug-resistant features with nosocomial pathogens and demanded regular screening in hospitals.

MIC of standard antibiotics

Based on the antibiotic sensitive assay, nosocomial bacterial as well as fungal species found to be multidrug resistance in features. It is essential to understand the MIC level of those.

***E. coli* strain DP1**

E. coli increasing antibiotic MIC noted with ofloxacin, levofloxacin, cefoperazone,

cefotaxime, ceftriaxone (1.56 µg/ml) > gentamycin 12.5 µg/ml > and amikacin (50 µg/ml). Hence drugs with MIC of 1.56 µg/ml are a better performer (Table 3 and Fig. 2).

***K. pneumoniae* strain DP2**

The best-scored MIC recorded with ofloxacin, levofloxacin, cefotaxime, ceftriaxone (1.56µg/ml) followed by amikacin, cefoperazone, and gentamycin (50 µg/ml) (Table 3 and Fig. 2).

***S. aureus* DP3**

The better MIC recorded with ofloxacin, levofloxacin, cefoperazone, cefotaxime, ceftriaxone (1.56 µg/ml) followed by gentamycin (12.5 µg/ml) and amikacin (25 µg/ml) (Table 3and Fig. 2).

***E. aerogenes* DP4**

The best MIC values for antibiotics recorded as levofloxacin (1.25 µg/ml), ofloxacin (3.15 µg/ml), cefotaxime (6.25 µg/ml), ceftriaxone (6.25 µg/ml), cefoperazone (12 µg/ml), amikacin (25 µg/ml) and gentamycin (25 µg/ml). Hence use of levofloxacin can be recommended (Table 3and Fig. 2).

***P. aeruginosa* DP5**

P. aeruginosa noted with best MIC value with levofloxacin (3.12 µg/ml), then cefoperazone (3.12 µg/ml) and cefotaxime (6.25 µg/ml);. At the same time, other drugs failed to give precise MIC values (Table 3and Fig. 2).

In a common point, MIC values for vancomycin, amphotericin and kanamycin not prominently recorded with any nosocomial pathogens.

***C. albicans* DP6**

The nosocomial fungal pathogen *C. albicans* DP6 noted for best MIC values with

voriconazole (6.25 µg/ml) and fluconazole (6.25 µg/ml) followed by caspofungin and ketoconazole (12.5 µg/ml). Thereby, high MIC noted with Itraconazole (50 µg/ml) as in Table 4.

Table.1 Antibiotic sensitivity pattern of nosocomial bacterial pathogens as per CLSI and EUCAST standard

Sr. No.	Name of Antibiotics	Zone of Inhibition (mm)				
		DP1	DP2	DP3	DP4	DP5
1	Ampicillin	21±1	-	35±1	18±2	10±2
2	Cefoperazone	29±1	10±3	28±1	15±2	17±2
3	Ceftriaxone	30±1	13±3	25±1	21±2	12±3
4	Nalidixic acid	22±2	13±1	12±1	12±2	-
5	Ciprofloxacin	31±2	21±1	26±1	21±1	-
6	Tetracycline	20±1	10±1	28±1	10±1	30±1
7	Erythromycin	16±2	11±1	27±2	12±2	15±1
8	Trimethoprim	22±1	17±1	22±2	27±2	-
9	Ofloxacin	25±2	18±2	24±1	17±2	-
10	Vancomycin	-	-	15±1	-	-
11	Amikacin	22±1	18±2	20±1	19±1	19±1
12	Gentamicin	20±2	13±1	20±1	12±1	-
13	Levofloxacin	26±2	18±1	25±1	20±2	-
14	chloramphenicol	22±2	15±1	23±2	11±2	23±1
Values in triplicate; ± zone deviation in mm; - No zone obtained						
DP1 <i>Escherichia coli</i> , DP2 <i>Klebsiella pneumoniae</i> , DP3 <i>Staphylococcus aureus</i> , DP4 <i>Enterobacter aerogenes</i> , DP5 <i>Pseudomonas aeruginosa</i>						

Table.2 Antibiotic sensitivity pattern of nosocomial fungal pathogens as per CLSI and EUCAST standard

Sr.No.	Name of Antibiotics	Zone of Inhibition (mm)
		<i>C. albicans</i> DP6
11	Variconazole	24±2
12	Caspofungin	19±2
13	Fluconazole	21±3
14	Ketaconazole	13±2
15	Intraconazole	-
Values in triplicate; ± zone deviation in mm; - No zone obtained		

Table.3 MIC assay of Standard drugs against Nosocomial pathogens

Sr.No.	Name of Antibiotics	Minimum Inhibitory Concentration in µg/ml				
		DP1	DP2	DP3	DP4	DP5
1	Ofloxacin 2mg/ml	1.56	1.56	1.56	3.15	-
2	Amikacin 125mg/ml	50	50	25	25	-
3	Levofloxacin 100mg/ml	1.56	1.56	1.56	1.56	3.12
4	Cefoperazone 100mg/ml	1.56	50	1.56	12.	3.12
5	Cefotaxime 100mg/ml	1.56	1.56	1.56	6.25	6.25
6	Vancomycin 100mg/ml	-	-	-	-	-
7	Amphotericin 100mg/ml	-	-	-	-	-
8	Kanamycin 100mg/ml	-	-	-	-	-
9	Ceftriaxone 100mg/ml	1.56	1.56	1.56	6.25	12.5
10	Gentamycin 25mg/ml	12.5	50	12.5	25	-

Table.4 MIC assay of Antifungal drugs against *C. albicans*

Sr. No.	Name of Antibiotics	Minimum Inhibitory Concentration in µg/ml
1	Variconazole 100mg/ml	6.25
2	Caspofungin 100mg/ml	12.5
3	Fluconazole 2mg/ml	6.25
4	Intrconazole 10 mg/ml	50
5	Ketaconazole 100mg/ml	12.5

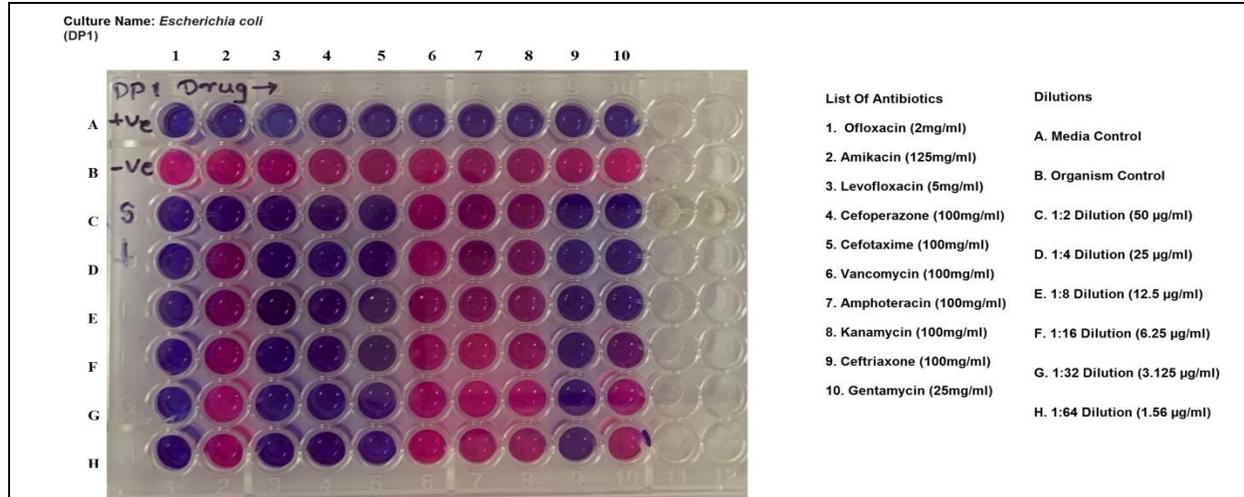


Fig 2a: Minimum inhibitory concentration assay of *Escherichia coli* (DP1) with antibiotics

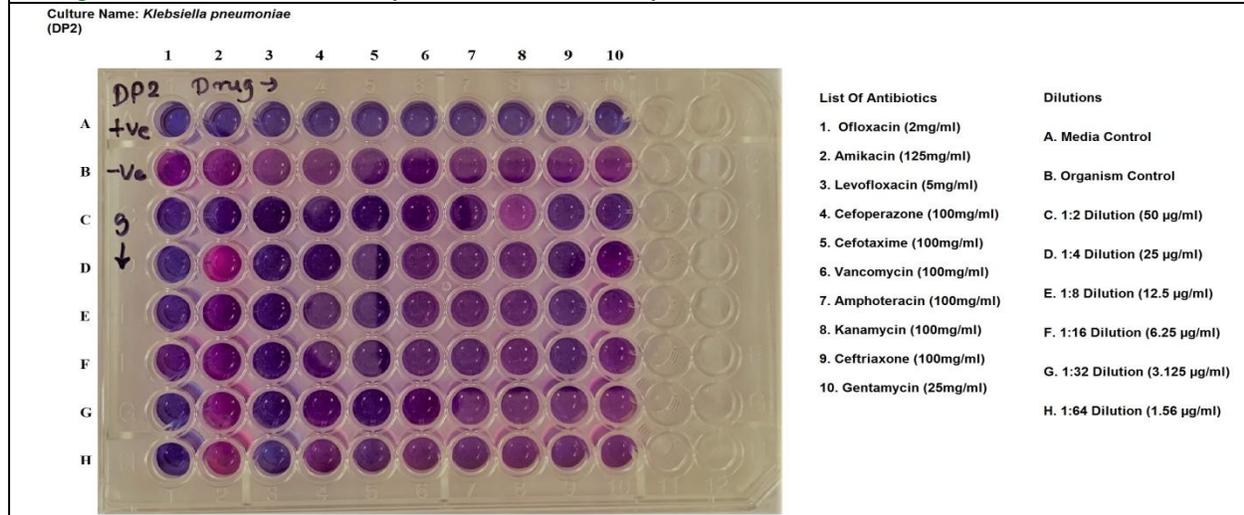


Fig 2b: Minimum inhibitory concentration assay of *Klebsiella pneumoniae* (DP2) with antibiotics

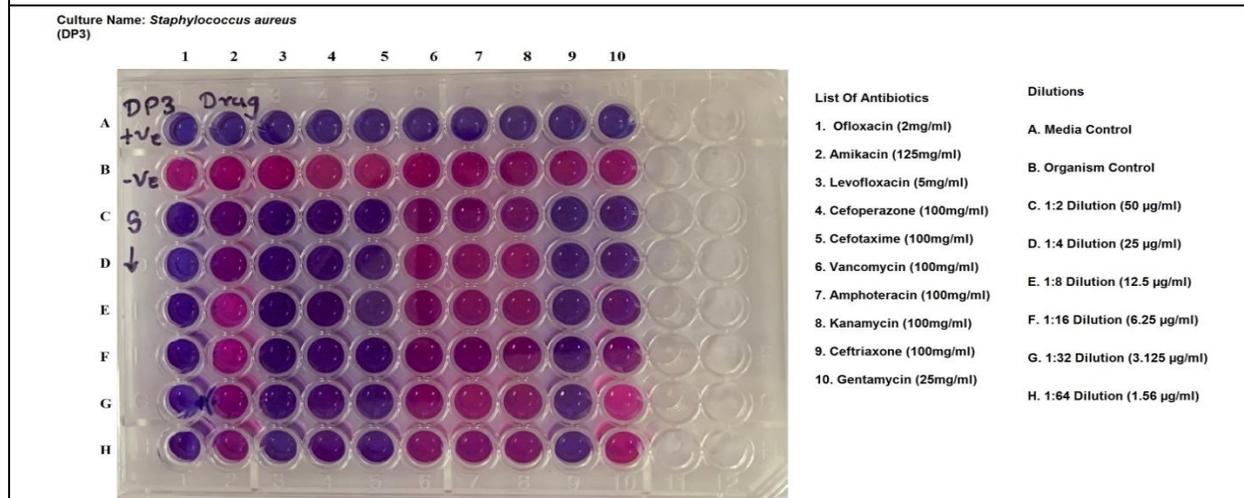


Fig 2c: Minimum inhibitory concentration assay of *Staphylococcus aureus* (DP3) with antibiotics

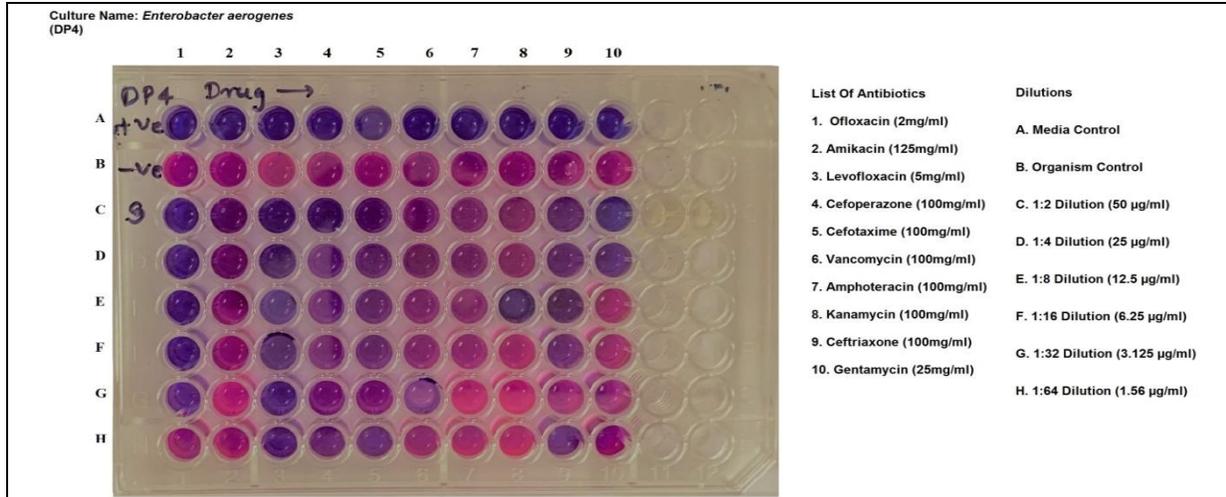


Fig 2d : Minimum inhibitory concentration assay of *Enterobacter aerogenes* (DP4) with antibiotics

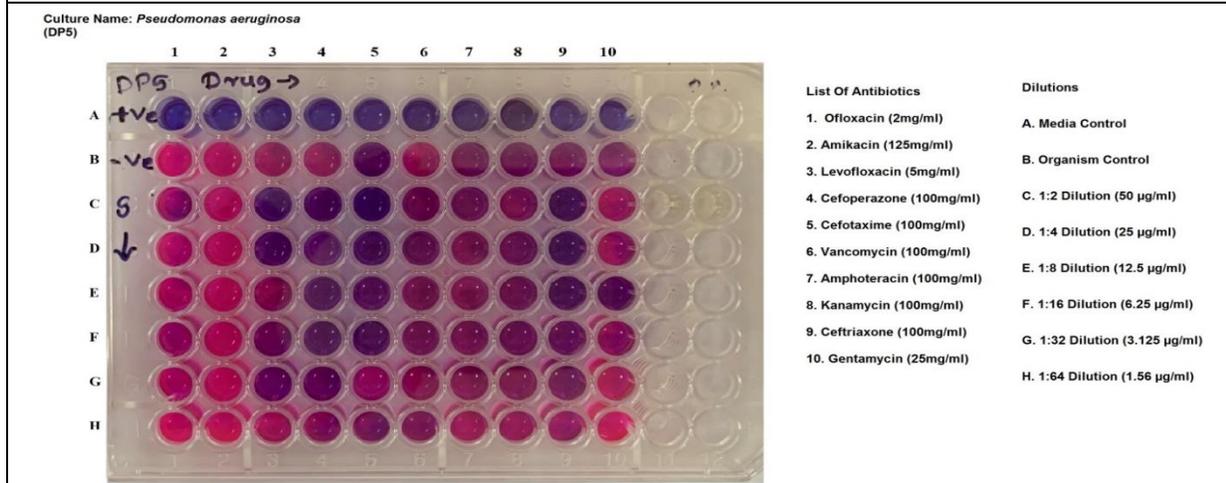


Fig 2e: Minimum inhibitory concentration assay of *Pseudomonas aeruginosa* (DP5) with antibiotics

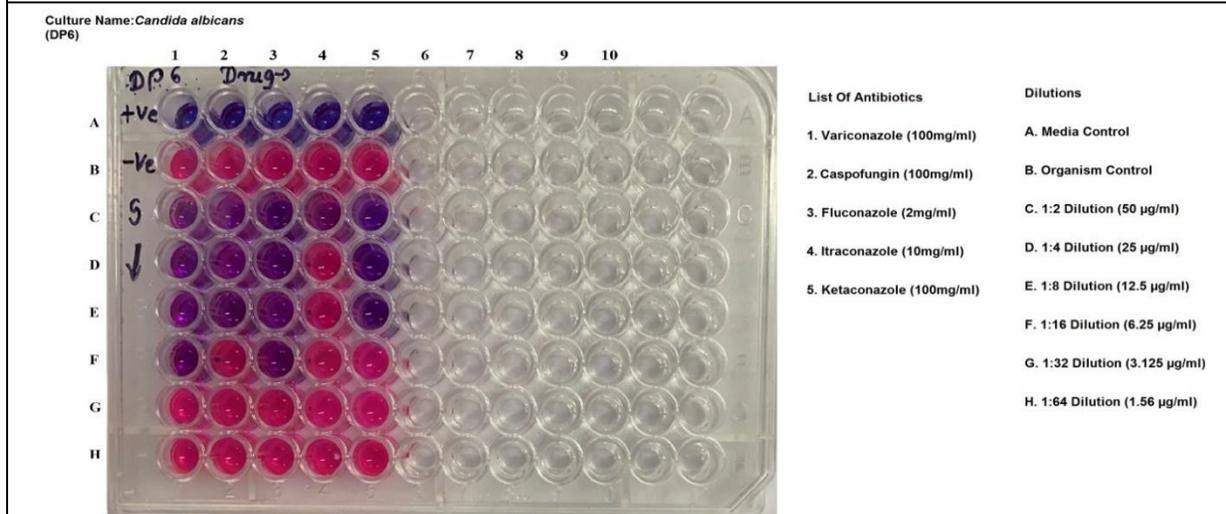


Fig 2f: Minimum inhibitory concentration assay of *C. albicans* (DP6) with antibiotics

The widespread of multidrug-resistant nosocomial pathogens in hospital settings reported in India; hence putting up an added burden for proper treatment by increasing the processes of screening, more extended hospital stay and increased economic burden (Yadav *et al.*, 2016; Mehta *et al.*, 2016; Jaggi *et al.*, 2013). In the present study, a private hospital of Parbhani situated in Maharashtra, India reported having an infection of nosocomial pathogens especially of *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecium* and *P. aeruginosa* as bacterial species. Further fungi species identified as *Candida albicans* as nosocomial pathogens. We found some strains possessed multidrug-resistant features once noted by antibiotic sensitivity assay. The study also recorded an increased MIC level to control better these pathogens, which is worrisome as per antibiotic therapy is concerned in earlier reports of multidrug resistance nosocomial pathogens very well reported in India. Pachori *et al.*, (2019) isolated MDR *P. aeruginosa* in intensive care unit; *MRSA S. aureus* (Kale and Dhawan, 2016); *E. coli* (Shaik *et al.*, 2017); *E. aerogenes* (Loiwal *et al.*, 1999), and Carbapenem-resistant *K. pneumoniae* (Veeraraghavan *et al.*, 2017). The study also noted that third-generation vancomycin resistance was also on a higher side, especially in the ICU and hospital ward samples mainly with *E. coli*, *K. pneumoniae*, *E. aerogenes* and *P. aeruginosa*. The drug resistance towards vancomycin by the *Enterococci* hence reported with chances of community hospital outbreaks (Beltrami *et al.*, 2000). The presence of *VRSA* and *MRSA* is also the common picture during hospital surveillance and putting up a series question about its use for treatment (Smith, 1999).

In the present study, isolated *C. albicans*, fortunately, reported sensitive towards voriconazole, caspofungin, and fluconazole but strong resistance recorded towards

Intraconazole and Ketoconazole. Earlier *C. albicans* resistance recorded prominently against intraconazole (Feng *et al.*, 2016; Giamarellos-Bourboulis *et al.*, 2019; Zida *et al.*, 2017). Ketoconazole resistance noted earlier in *C. albicans* in Indian clinical isolates (Mane *et al.*, 2016). About 22.9% of strains of *C. albicans* reported ketoconazole resistance in Ouagadougou and linked to be showcase possibility of community transmissions.

In conclusion, as per the present study, it has come to fore that hospital in India need to screen regularly for the prevalence status of nosocomial infection so that community transmission could be avoided and better treatment could ensure by establishing permanent surveillance body as multidrug resistant nosocomial pathogens were prevalent in the hospital screened.

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