ARTICLE
Infectious Diseases Research 2023;4(2):8. https://doi.org/10.53388/IDR2023008

Effect of picking multiple colonies on antimicrobial susceptibility diagnostic outcome in a clinical bacteriology setting

Bhoj R Singh[1*], Dharmendra K Sinha[1], Varsha Jayakumar[1], Himani Agri[1], Akanksha Yadav[1], Ravichandran Karthikeyan[1]

[1]Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar 243 122, India.

[*]**Corresponding to:** Bhoj R Singh, Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar 243 122, India. E-mail: brs1762@gmail.com.

Author contributions

Bhoj R Singh contributed to planning, execution, bacteriological work, analysis, and manuscript writing, while Dharmendra K Sinha contributed to analysis and manuscript preparation. Varsha Jayakumar and Himani Agri performed bacteriological analysis of samples and antibiotic susceptibility assays, while Akanksha Yadav and Ravichandran Karthikeyan determined ESBL and MBL production.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This work has been supported by ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India, under an ongoing service project (IVRI-Epid/2014-25).

Abbreviations

AST, antimicrobial susceptibility testing; ESBL, extended-spectrum β-lactamase; MBL, Metallo-β-lactamase; MIC, minimum inhibitory concentration; EDTA, ethylene-diamine tetra-acetic acid.

Citation

Singh BR, Sinha DK, Jayakumar V, Agri H, Yadav A, Karthikeyan R. Effect of picking multiple colonies on antimicrobial susceptibility diagnostic outcome in a clinical bacteriology setting. *Infect Dis Res.* 2023;4(2):8. doi: 10.53388/IDR2023008.

Executive editor: Na Liu.

Received: 25 November 2022; **Accepted:** 02 March 2023; **Available online:** 21 March 2023.

Abstract

Background: It is often taught in clinical microbiology to pick up multiple colonies for giving results of antimicrobial susceptibility testing (AST) even if the infection is detected due to a single type of bacteria. However, what should be the minimum number of colonies? The clinical laboratory manuals are silent and give different numbers. Thus, the present study was conducted to evaluate the impact of picking up more than one colony for antimicrobial susceptibility. **Method:** The study was conducted on those clinical samples yielding *Escherichia coli* either as pure culture or as a prominent cause of infection. The impact of testing multiple colonies of *Escherichia coli* isolates was assessed on AST results through the detection of extended-spectrum β-lactamase (ESBL) producing and Metallo-β-lactamase (MBL) producing *Escherichia coli* carriage in clinical samples. **Results:** A total of 1031 samples having *E. coli* as the most prominent or single pathogen were analyzed. It included testing either one, two, three, four, five, or more than five (6-10) representative *E. coli* colonies from 526, 247, 115, 76, 31, and 36 samples, respectively. The study revealed that testing of less than three representative colonies significantly ($p < 0.05$) impacts the outcome. However, the best outcome was when ≥ 6 representative colonies were tested for AST. **Conclusion:** The study suggested that ≥ 6 representative colonies should be emulsified for antimicrobial susceptibility tests not to miss possible infection with ESBL or MBL-producing mutants, even in mono-culture infections.

Keywords: ESBL; MBL; clinical-diagnosis; AST; antibiotics; antimicrobials

Infectious Diseases Research 2023;4(2):8. https://doi.org/10.53388/IDR2023008

Introduction

For most chronic and life-threatening infections, antibiotic susceptibility testing (AST) of the infecting organism is recommended for successful treatment [1-4]. The reference manuals and books on Clinical Microbiology [1-4] suggest picking up pure and isolated multiple colonies for the identification of microbes and giving causal diagnosis and also for suggesting antimicrobial drugs after antimicrobial susceptibility tests (AST). Most of the clinical microbiology manuals [1-4] suggest picking up a representative colony when you suspect infection with a single microbe on the basis of the growth of microbes on the culture plates. Some manuals on AST procedures suggest taking 3-5 well-isolated colonies of the same morphological type to make suspensions for conducting the test [5]. The CAESAR Manual [6] suggests collecting several morphologically similar colonies for doing AST, but the exact number is not specified. The guidelines by CLSI on conducting AST [7] suggest emulsifying a 1-µL loopful of bacteria from an overnight blood agar plate. However, it is almost a vague statement as a *Klebsiella* spp. A colony can fill several loops while filling the loop with the growth of *Streptococcus* spp. strains, you may need dozens of colonies. In normal laboratory practice, usually, one representative colony is picked up for AST if the infection is suspected by a single type of bacteria to save time, labor and funds. To understand the impact of picking multiple colonies on identifying extended spectrum β-lactamase (ESBL) and Metallo β-lactamase (MBL) producer *Escherichia coli* carrying samples has been assessed in the present study. The reference ATCC-2469, ATCC-35218, and ATCC-25922 *E. coli* strains were used as ESBL positive, MBL (NDM-1) positive and antibiotic susceptible controls, respectively.

Materials and methods

All those samples submitted to the Clinical Epidemiology Laboratory were processed for isolation and identification of bacteria using the standard protocol recommended for clinical laboratories [1]. From the samples having *E. coli* as the prominent or single type of bacteria on the inoculated plates, colonies were randomly chosen to pick only one (Monday samples), two (Tuesday samples), three (Wednesday samples), four (Thursday samples), five (Friday samples), and more than five (6-10, Saturday samples) representative colonies of *E. coli*. A total of 1031 samples having *E. coli* as the most prominent or single pathogen were included in the study. Only one, two, three, four, five and more than five (6-10) representative *E. coli* colonies were picked up from 526, 247, 115, 76, 31 and 36 samples, respectively for AST, ESBL and MBL production.

All *E. coli* isolates were tested for purity on re-streaking on 5% sheep blood agar and only purified colonies were tested with E-strip for detection of ESBL and MBL production following ESBL and MBL E-strips supplier (BioMerieux, Marcy-l'Étoile, France) guidelines. The results were interpreted as per CLSI guidelines [7]. An *E. coli* isolate were considered ESBL producer when there were four-fold reductions in minimum inhibitory concentration (MIC) of cefotaxime/ceftazidime/cefepime in the presence of clavulanic acid/tazobactam using ESBL CT/CTL 16/1, ESBL TZ/TZL 32/4 and ESBL PM/PML 16/4 E-strips. Similarly, *E. coli* isolates having a 4-fold reduction in MIC of meropenem in the presence of ethylene-diamine tetra-acetic acid (EDTA) using MBL MP/MPI 8/2 (BioMerieux) were designated as MBL producers.

The data of ESBL and MBL production was analyzed using MS Office Excel-2007 applying Chi-Square Test.

Results and discussion

The results of the detection of ESBL and MBL producer *E. coli* carrying samples (Table 1) revealed that of the 1031 samples in the study 618 (59.94%) and 212 (20.56%) samples had ESBL and MBL producer *E. coli*, respectively.

Table 1 Number of colonies checked and number of samples positive for extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing *Escherichia coli* (year-wise distribution)

Colonies picked	Determinants	Year of number of samples tested and positive for ESBL and MBL production												Total
		2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	
1	Samples	49	48	42	23	30	53	69	69	70	30	33	10	526
1	ESBL	11	17	20	15	15	32	45	35	24	17	23	4	258
1	MBL	8	0	3	5	7	8	8	18	16	2	3	2	80
2	Samples	14	8	15	1	7	27	40	40	33	27	26	9	247
2	ESBL	5	5	12	1	1	22	28	30	16	17	18	6	161
2	MBL	2	0	1	0	5	6	5	4	16	2	5	4	50
3	Samples	3	6	1	1	4	9	8	34	19	15	12	3	115
3	ESBL	2	2	0	1	4	9	7	25	16	12	5	3	86
3	MBL	0	0	0	0	3	0	2	6	10	6	2	1	30
4	Samples	2	1	1	0	3	6	9	19	11	11	11	2	76
4	ESBL	1	1	1	0	3	6	7	11	9	11	8	2	60
4	MBL	1	0	0	0	1	2	2	4	7	3	6	0	26
5	Samples	0	1	1	0	1	4	2	12	2	5	3	0	31
5	ESBL	0	0	1	0	1	4	1	10	1	4	1	0	23
5	MBL	0	0	1	0	0	1	0	4	0	2	2	1	11
6	Samples	0	1	0	0	1	0	1	5	4	3	0	1	16
6	ESBL	0	1	0	0	1	0	1	5	3	3	0	1	15
6	MBL	0	0	0	0	1	0	1	2	2	0	0	0	6
7	Samples	0	0	0	0	0	0	1	2	1	1	1	0	6
7	ESBL	0	0	0	0	0	0	0	1	0	1	0	0	2

Table 1 Number of colonies checked and number of samples positive for extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing *Escherichia coli* (year-wise distribution) (*continued*)

Colonies picked	Determinants	Year of number of samples tested and positive for ESBL and MBL production												Total
		2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	
7	MBL	0	0	0	0	0	0	0	0	0	0	1	0	1
8	Samples	0	0	0	0	0	0	0	1	2	1	0	1	5
8	ESBL	0	0	0	0	0	0	0	1	1	1	0	1	4
8	MBL	0	0	0	0	0	0	0	1	2	0	0	1	4
9	Samples	0	0	0	0	0	0	0	0	0	0	3	0	3
9	ESBL	0	0	0	0	0	0	0	0	0	0	3	0	3
9	MBL	0	0	0	0	0	0	0	0	0	0	1	0	1
10	Samples	0	0	0	0	0	0	0	0	0	1	1	0	2
10	ESBL	0	0	0	0	0	0	0	0	0	1	1	0	2
10	MBL	0	0	0	0	0	0	0	0	0	0	1	0	1
11	Samples	0	0	0	0	0	0	0	0	0	1	0	0	1
11	ESBL	0	0	0	0	0	0	0	0	0	1	0	0	1
11	MBL	0	0	0	0	0	0	0	0	0	1	0	0	1
13	Samples	0	0	0	0	0	0	1	0	0	0	0	0	1
13	ESBL	0	0	0	0	0	0	1	0	0	0	0	0	1
13	MBL	0	0	0	0	0	0	0	0	0	0	0	0	0
15	Samples	0	0	0	0	0	0	0	0	0	0	1	0	1
15	ESBL	0	0	0	0	0	0	0	0	0	0	1	0	1
15	MBL	0	0	0	0	0	0	0	0	0	0	0	0	0
19	Samples	0	0	0	0	0	0	0	0	0	1	0	0	1
19	ESBL	0	0	0	0	0	0	0	0	0	1	0	0	1
19	MBL	0	0	0	0	0	0	0	0	0	1	0	0	1

Further analysis revealed (Figure 1) that if you are picking only one, two, three, four and five representative colonies for ESBL and MBL production detection, you have 34.28, 18.15, 8.55, 4.38, and 2.68 percent chances of missing detection of ESBL producer *E. coli* carrying samples. There were 26.46, 21.43, 15.58, 7.46, and 6.19 percent chances of missing detection of carbapenem-resistant MBL-producing *E. coli* carrying samples, respectively, on picking 1, 2, 3, 4, and 5 colonies in comparison to the picking of six or more representative colonies. For detecting samples with MBL-positive *E. coli,* picking up one or two colonies had no significant difference ($p > 0.05$) but when ≥ 3 colonies were picked up impact was highly significant ($p < 0.01$). However, for the detection of ESBL *E. coli* difference was significant ($p < 0.01$) between picking one and two colonies. It was evident that picking more colonies from a sample was always associated with the detection of a higher number of ESBL and MBL-positive *E. coli* samples. However, there was no significant difference ($p > 0.05$) in results when ≥ 3 colonies were picked up. The study suggests that even when only a single type of bacteria is suspected to be the cause of the infection, it is not advisable to test less than three colonies for determining the AST of bacteria from clinical samples. Picking less number of colonies may result in missed ESBL and MBL positive strains. The results may severally impact the therapeutic outcome of antimicrobial therapy even after AST. Testing three or more colonies for AST will certainly increase the cost of diagnostic testing. To cut the diagnosis cost, it may be advised that instead of emulsifying a single or a few representative colonies [1-4] or a loopful of bacteria [7] or 3-5 colonies [5], ≥ 6 colonies be picked up to emulsify for AST. The suggested procedure may keep the cost at the same scale without compromising accuracy of AST results for clinical samples. It is a known fact that all progeny bacteria of a single clone resistant to a particular antibiotic may not be equally resistant. It is especially common when antimicrobial resistance is mediated through plasmids or other mobile genetic elements (R-factors) due to the ongoing natural process of mutation and need of the bacteria to maintain the R-factors [8].

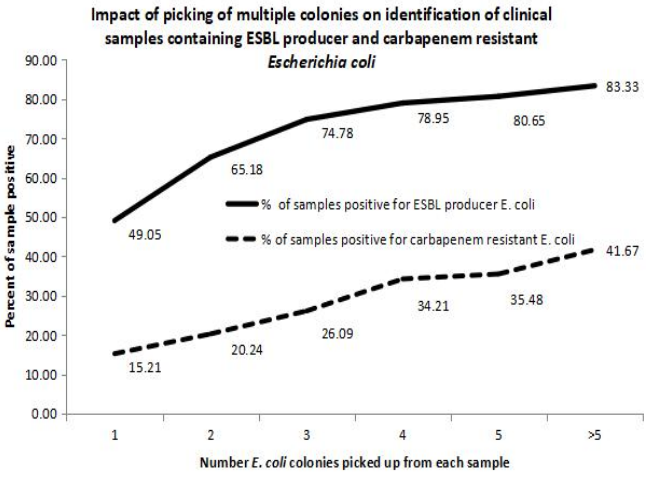

Figure 1 Impact of picking of multiple colonies on identification of clinical samples containing ESBL producer and carbapenem resistant *Escherichia coli*

References

1. Barrow GI, Feltham RKA, eds. *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge University Press; 1993. http://doi.org/10.1017/CBO9780511527104
2. Hunter-Cevera JC, Belt A. Isolation of Cultures. In: Demail AL, Davies JE, Atlas RM, Cohen G, Hershberger CL, Hu SH, Sherman H, Wilson RC, Wu JHD, ed. *Manual of Industrial Microbiology and Biotechnology*. 2nd ed. Washington DC, USA: American Society for Microbiology Press; 1999.
3. Kolmer JA, Sapaulding EH, Robinson HW. *Approved Laboratory Technic*. 5th ed. Indian ed. NewYork, USA: Appelton-Century-Crofts Inc; 1969.
4. Levinson SA, MacFate RP. Clinical laboratory diagnosis. 7th ed. Philadelphia, USA: *Lea and Febiger*; 1969.
5. Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. *Standard Operating Procedures. Antimicrobial Resistance in Priority Bacterial Pathogens National AMR Surveillance Network (NARS-Net). National Programme on Antimicrobial Resistance Containment.* New Delhi, India: National Centre for Disease Control; 2021.
6. Central Asian and European Surveillance of Antimicrobial Resistance. CAESAR Manual. Version 3.0. Geneva: WHO Regional Office for Europe; 2019. http://doi.org/WHO/EURO:2019-3583-43342-60804
7. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing, M100*. 31st ed. Wayne, USA: Clinical and Laboratory Standards Institute; 2021.
8. Kumar S, Singh BR. An overview on mechanisms and emergence of antimicrobials drug resistance. *Adv Anim Vet Sci* 2013;1(2S):7–14. http://www.nexusacademicpublishers.com/journal/4