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ORIGINAL ARTICLE

JMHRS
JOURNAL OF MODERN HEALTH AND REHABILITATION SCIENCES

Exploring the Antibiotic Resistance Profile and blaOXA-23 Sequence Analysis in Acinetobacter Baumannii Isolates from a Tertiary Care Hospital, Lahore, Pakistan

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ABSTRACT

Background: *Acinetobacter baumannii* is a major cause of healthcare associated infections, particularly in intensive care units, and exhibits extensive resistance to multiple antibiotic classes including carbapenems.

Objective: To determine the antibiotic resistance profile, biofilm formation, and prevalence of the blaOXA-23 gene among *A. baumannii* isolates from a tertiary care hospital in Lahore, Pakistan.

Methods: A total of 150 clinical specimens (pus, sputum, and blood) were collected from the surgical ICU over a three month period. Isolates were identified using API-20E, polymerase chain reaction (PCR), and Sanger sequencing. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method in accordance with CLSI (2023) standards. Biofilm formation was quantified using the microtiter plate assay. Detection and sequence analysis of blaOXA-23 were conducted through PCR amplification and phylogenetic comparison with regional strains.

Results: Out of 150 samples, 50 (33.3%) yielded *A. baumannii*. Twenty isolates (40%) demonstrated biofilm formation, and 19/20 (95%) of these carried blaOXA-23. Overall, blaOXA-23 was present in 19/50 (38%) isolates. Complete resistance (100%) was observed to meropenem and cephradine, while imipenem and amikacin resistance reached 75%. One XDR isolate harboring blaOXA-23 showed 96–98% genetic similarity with regional sequences.

Conclusion: The high frequency of blaOXA-23 among biofilm-forming *A. baumannii* isolates underscores an alarming trend in carbapenem resistance and necessitates stringent infection control practices and antibiotic stewardship in tertiary care settings.

Keywords: *Acinetobacter baumannii,* Antimicrobial Susceptibility, blaOXA-23, Biofilm, Carbapenem Resistance.

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Disclaimers
Conflict of Interest: None declared
Data/Supplements: Available on request.
Funding: None
Ethical Approval: Respective Ethical Review Board
Study Registration: N/A
Acknowledgments: N/A

Article Info
Received: 22 September 2025, *Accepted:* 01 November 2025,
Published Online: 12 November 2025

How to Cite: Babar M, Suleman MM, Zahra N, Naveed H. Exploring the Antibiotic Resistance Profile and blaOXA-23 Sequence Analysis in *Acinetobacter baumannii* Isolates from a Tertiary Care Hospital, Lahore, Pakistan. J Mod Health Rehab Sci. 2025;2(4):186.
Available from: https://jmhrs.com/index.php/jmhrs/article/view/186

Introduction

Acinetobacter baumannii (A. baumannii) is a Gram-negative, non-fermentative, opportunistic bacterium belonging to the genus Acinetobacter (1). It has gained increasing attention in recent years as one of the most significant causes of healthcare associated infections, particularly in intensive care units (ICUs) (2). This pathogen exhibits remarkable environmental persistence and genetic adaptability, enabling it to survive under desiccated conditions and resist multiple antimicrobial agents. The clinical relevance of *A. baumannii* lies in its capacity to cause a broad range of infections, including ventilator associated pneumonia, bloodstream infections, wound infections, and meningitis, particularly in immunocompromised or critically ill patients (3). Understanding its transmission dynamics and molecular mechanisms of resistance is vital to designing targeted interventions and optimising therapeutic strategies.

The World Health Organization (WHO) has classified *A. baumannii* as a multidrug resistant (MDR) priority pathogen due to its ability to develop resistance to several antibiotic classes (4). It exhibits resistance to β-lactams such as penicillins and cephalosporins, as well as to fluoroquinolones, aminoglycosides, and carbapenems, which are typically considered last line options for treating Gram-negative infections. This resistance arises from multiple molecular mechanisms, including the production of β-lactamases that degrade β-lactam antibiotics (5), overexpression of efflux pumps that expel antimicrobial compounds from bacterial cells (6), alterations in outer membrane porin proteins reducing permeability (7), and horizontal transfer of antibiotic resistance genes between bacterial strains (8).

In addition to these acquired mechanisms, *A. baumannii* possesses intrinsic resistance due to its unique chromosomal structure and regulatory pathways (9), further complicating therapeutic management and increasing the risk of treatment failure (10). Because of these multidrug resistant features, infections caused by *A. baumannii* often require combination therapy or the use of less effective antimicrobial agents, leading to prolonged hospital stays, higher healthcare costs, and increased mortality (11, 12). The recognition of *A. baumannii* as a high priority MDR pathogen emphasizes the necessity for continuous epidemiological surveillance, stringent infection control practices, and the development of novel antimicrobial compounds to limit its spread (13, 14).

In Pakistan, the prevalence of *A. baumannii* infections follows trends observed globally, with increasing isolation rates from ICUs and tertiary care hospitals (15). Of particular concern is the emergence of carbapenem resistance, which is mediated by several mechanisms that collectively reduce the efficacy of carbapenem antibiotics (16). These mechanisms include the production of carbapenemase enzymes, particularly OXA-type β-lactamases encoded by the blaOXA-23 gene, which hydrolyze the β-lactam ring and inactivate the drug (17). Resistance may also result from changes in porin proteins that alter membrane permeability, efflux pump hyperactivity that decreases intracellular antibiotic concentration, and structural alterations in penicillin binding proteins (PBPs) that impair antibiotic binding (18, 19). The coexistence of these mechanisms, alongside other resistance determinants such as aminoglycoside modifying enzymes, contributes to the development of extensively drug resistant (XDR) strains (20).

The growing prevalence of carbapenem resistant *A. baumannii* in clinical settings represents a major public health challenge. Investigating molecular determinants such as the blaOXA-23 gene, together with biofilm forming capabilities, is essential for understanding the pathogenic potential of this organism and formulating evidence based infection prevention and antimicrobial stewardship strategies.

Materials and Methods

Clinical specimens, including pus, sputum, and blood, were collected from patients admitted to the surgical intensive care unit (ICU) of a tertiary care hospital in Lahore, Pakistan, over three months. A total of 150 samples were obtained and processed according to standard microbiological procedures. The specimens were cultured on MacConkey agar plates and incubated at 37°C for 24 hours. Colonies exhibiting morphological characteristics consistent with those of Acinetobacter species were subjected to Gram staining and further identified through biochemical testing using the API-20E identification system (bioMérieux, France), following the manufacturer’s instructions.

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (21). The antibiotics tested included meropenem, imipenem, cephradine, amikacin, tobramycin, ciprofloxacin, and levofloxacin. Biofilm formation was assessed using the standard crystal violet microtiter plate assay. After incubation and staining, optical density (OD) was measured at 570 nm, and isolates were classified as non, weak, moderate, or strong biofilm producers based on OD cut off values.

Genomic DNA was extracted from overnight bacterial cultures using the GeneAll® Exgene™ DNA extraction kit (Cat. No. 106-101). The polymerase chain reaction (PCR) was performed in a 20 µL reaction mixture containing 10 µL of 2X Master Mix (Bioshop), 0.5 µL each of forward and reverse primers, 2.5 µL of template DNA, and 6.5 µL of nuclease free water, yielding a final volume of 20 µL. The specific primers used for amplification included were (Table 1):

Table 1: Primer used for PCR Amplification

Target Gene	Primer Sequence (5′→3′)	Amplicon Size (bp)
blaOXA-23	F: GATCGGATTGGAGAACCAGA	510
	R: ATTTCTGACCGCATTTCCAT	
ISAba1	F: ATGCAGCGCTTCTTTGCAGG	393
	R: AATGATTGGTGACAATGAAG	

Amplified PCR products were resolved on 2% agarose gel electrophoresis stained with ethidium bromide and visualized under ultraviolet illumination. PCR products were purified using the WizPrep™ Gel/PCR Purification Mini Kit (W70150-300) and subjected to Sanger sequencing. The resulting chromatograms (AB1 format) were inspected, trimmed, and assembled into consensus sequences using BioEdit software. Sequence identity was confirmed by BLAST analysis through the NCBI database. Multiple sequence alignment and phylogenetic analysis were performed using Clustal Omega software to determine genetic relatedness among isolates. Accession numbers were assigned for sequences submitted to the NCBI GenBank database.

Results

A total of 150 clinical specimens were processed during the study period, out of which 50 isolates of Acinetobacter baumannii (*A. baumannii*) were recovered, representing a culture positivity rate of 33.3%. Among these isolates, biofilm formation and the presence of resistance genes were evaluated. Out of the 50 *A. baumannii* isolates, 20 (40%) demonstrated biofilm forming ability. Nineteen of these biofilm producing isolates (95%) were positive for the blaOXA-23 gene, while the blaOXA-23 gene was detected in 19 out of 50 isolates overall (38%). The insertion sequence ISAba1 was also observed among blaOXA-23 positive isolates, consistent with previously reported findings. One extensively drug resistant (XDR) isolate was selected for sequencing and subsequent phylogenetic analysis (Figure 1).

PCR amplification confirmed the presence of blaOXA-23 (~510 bp) and ISAba1 (~393 bp) in positive isolates. Figure 1 illustrates the agarose gel electrophoresis results, where lane 1 represents the positive control (16S rRNA), lane 2 shows an ISAba1 positive isolate, lane 3 indicates a blaOXA-23 positive isolate, and lane 4 serves as the no-template control (Figure 2). Antimicrobial susceptibility testing demonstrated high resistance levels to most tested antibiotics. All isolates (100%) were resistant to meropenem and cephradine, while 75% were resistant to imipenem and amikacin. Variable sensitivity was observed toward fluoroquinolones and aminoglycosides, with tobramycin showing the highest sensitivity rate (75%) among all tested agents. A summary of antimicrobial susceptibility results is presented (Figure 3).

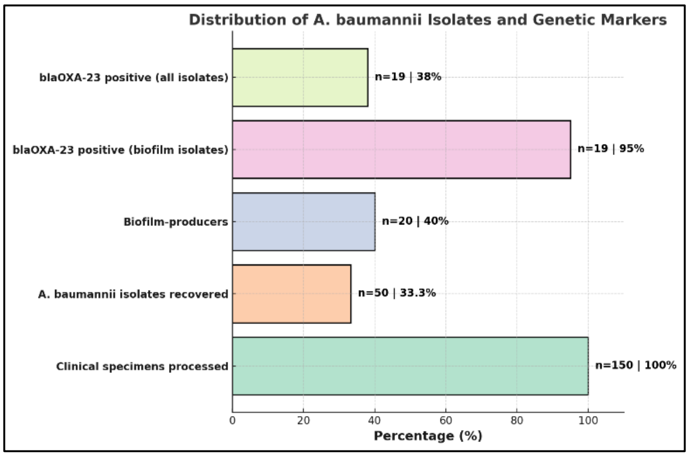


Figure 1: Distribution of Clinical Specimens, *A. baumannii* Isolates, Biofilm Formation, and blaOXA-23 Detection

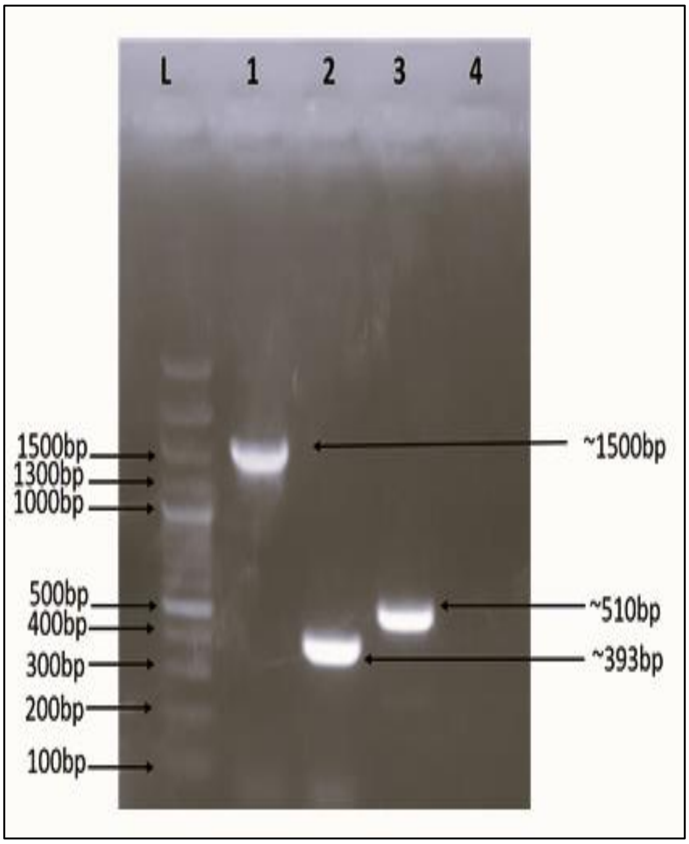


Figure 2: Agarose gel electrophoresis showing PCR products for ISAba1 (~393 bp) and blaOXA-23 (~510 bp). L = 100-bp ladder; lane 1 = positive control (16S rRNA), lane 2 = ISAba1-positive isolate, lane 3 = blaOXA-23 positive isolate, lane 4 = no-template control.

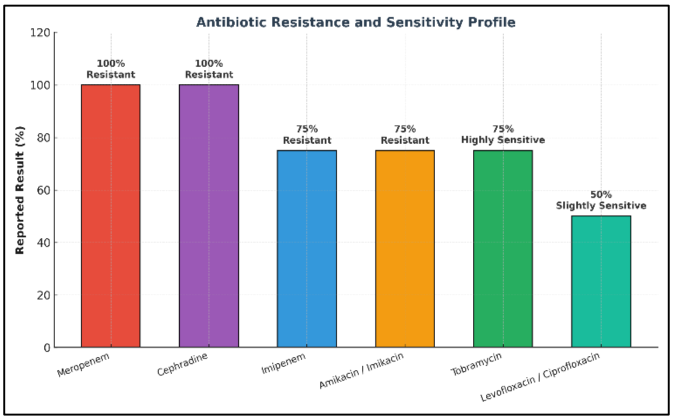
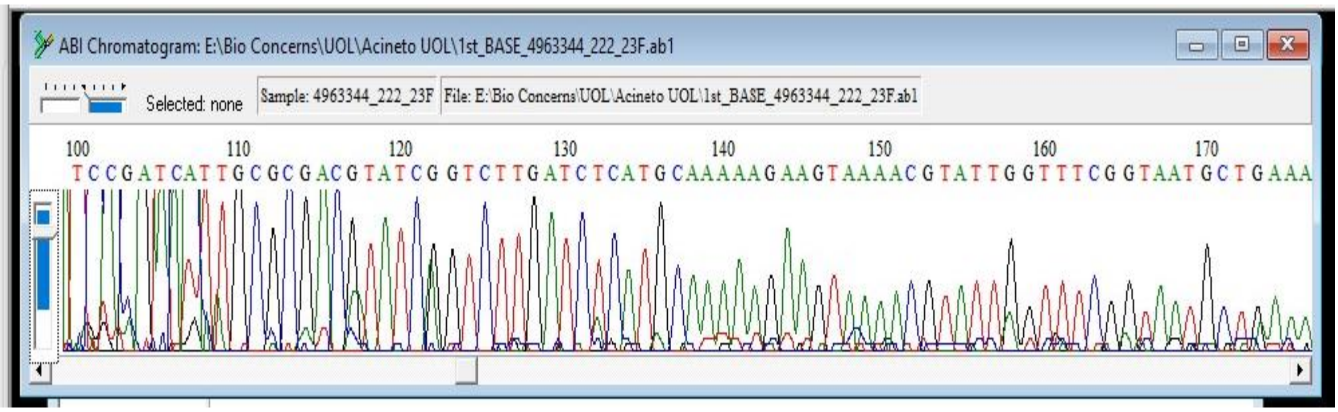


Figure 3: Antimicrobial Resistance and Sensitivity Distribution among *A. baumannii* Isolates

For sequencing analysis, the blaOXA-23 gene was purified using the WizPrep™ Gel/PCR Purification Mini Kit (Cat. No. W70150-300). The purified PCR product was sequenced, and results were obtained as AB1 chromatogram files and FASTA sequences. The chromatograms were analyzed and trimmed using BioEdit software to generate high quality consensus sequences (Figure 4). The verified sequences were subjected to NCBI BLAST for homology confirmation and species identification.

Phylogenetic analysis was conducted using the Clustal Omega online tool for multiple sequence alignment and the neighbor joining method to construct the phylogenetic tree. The comparative analysis revealed two distinct clades. Clade 1 showed 96–98% sequence similarity with *A. baumannii* strains previously reported from neighboring countries, while Clade 2 represented isolates unique to Pakistan. These Pakistani isolates demonstrated novel sequence characteristics not previously reported in other global submissions, and the respective sequences will be deposited in the NCBI GenBank database (Figure 5). Overall, the findings confirmed a high prevalence of carbapenem resistant *A. baumannii* (CRAB) strains harboring blaOXA-23 in this tertiary care hospital, highlighting significant genetic diversity and a potential emerging lineage unique to Pakistani isolates.

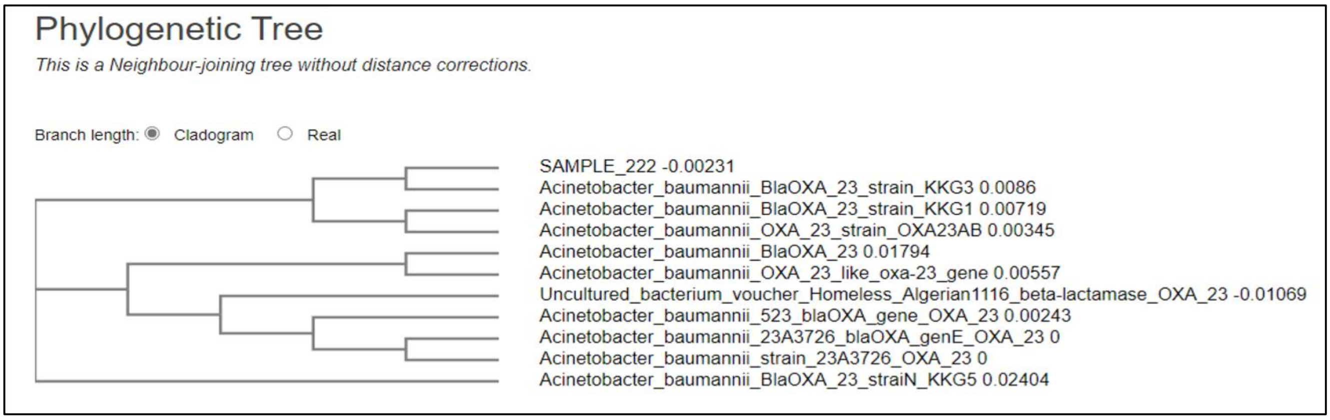

ABI Chromatogram: E:\Bio Concerns\UOL\Acineto UOL\1st_BASE_4963344_222_23F.ab1

Selected: none

Sample: 4963344_222_23F

File: E:\Bio Concerns\UOL\Acineto UOL\1st_BASE_4963344_222_23F.ab1

Figure 4: Bioinformatic Analysis by BioEdit


Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Branch length: ● Cladogram ○ Real

Figure 5: ***Neighbor joining phylogenetic tree of*** **blaOXA-23** ***sequences obtained in this study compared with representative regional reference sequences. Bootstrap support values ≥ 70% are shown at the***

Discussion

This study investigated the antimicrobial resistance profile, biofilm forming potential, and blaOXA-23 gene carriage among *Acinetobacter baumannii* (*A. baumannii*) isolates obtained from the surgical intensive care unit (ICU) of a tertiary care hospital in Lahore. The recovery rate of *A. baumannii* was 33.3% (50/150), indicating a substantial burden of this pathogen in the clinical setting. These findings were consistent with previous reports that have identified *A. baumannii* as a major cause of healthcare associated infections, particularly in critical care units, where invasive procedures and prolonged hospital stays increase the risk of colonization and infection. The high isolation frequency observed in this study reflected the ongoing challenges of infection control within Pakistani hospitals and reinforced the global concern regarding the rise of multidrug resistant *A. baumannii*.

Antimicrobial susceptibility testing revealed alarming levels of resistance to commonly used antibiotics. All isolates were resistant to meropenem and cephradine, while 75% demonstrated resistance to imipenem and amikacin. These findings closely aligned with data from other centers in Pakistan and neighboring South Asian countries, where carbapenem resistance in *A. baumannii* has been reported in 70–90% of isolates (21). The variable sensitivity patterns to fluoroquinolones and aminoglycosides, with tobramycin exhibiting 75% sensitivity, indicated that therapeutic options remain severely limited. Such extensive resistance profiles underscored the clinical importance of carbapenem resistant *A. baumannii* (CRAB), which has been recognized by the World Health Organization (WHO) as a critical priority pathogen for the development of new antimicrobials.

Biofilm formation was observed in 40% of isolates, among which 95% were positive for the blaOXA-23 gene. The strong association between biofilm formation and blaOXA-23 carriage suggested that biofilm producing isolates might serve as key reservoirs for carbapenem resistance genes. These results were consistent with earlier research showing that biofilm growth enhances bacterial survival on hospital surfaces and medical devices, thereby facilitating persistence and cross transmission in healthcare environments (22). The detection of blaOXA-23 in 38% of all isolates further supported its role as one of the dominant carbapenemase genes in Pakistan, in accordance with previous reports from South Asia describing blaOXA-23 as a major contributor to carbapenem resistance in clinical *A. baumannii* isolates.

Molecular sequencing and phylogenetic analysis of the blaOXA-23 gene provided additional insights into the genetic relatedness of the isolates. The sequenced gene from the extensively drug resistant (XDR) isolate formed two distinct clades, with clade 1 showing 96–98% similarity to strains previously identified in neighboring countries. This suggested possible regional dissemination of resistant clones through patient movement or environmental persistence. The discovery of clade 1 variants unique to Pakistani isolates pointed toward potential local evolutionary divergence or independent acquisition of resistance elements. Such molecular characterization was essential for understanding the epidemiological linkages of *A. baumannii* strains and for designing effective infection control strategies.

The clinical implications of these findings were substantial. The high prevalence of resistance across multiple antibiotic classes, including carbapenems, left clinicians with limited therapeutic alternatives. The emergence of *A. baumannii* strains resistant to both first line and reserve antibiotics could result in treatment failure, prolonged hospitalization, and increased healthcare costs. These outcomes emphasized the urgent need for antimicrobial stewardship programs focusing on the rational use of antibiotics, regular monitoring of resistance trends, and implementation of stringent infection prevention and control measures. Furthermore, the potential utility of combination therapies or newer antimicrobial agents such as colistin, tigecycline, and β-lactamase inhibitors should be explored for managing CRAB infections where feasible.

Despite the valuable insights provided, this study had certain limitations. The relatively small sample size and the single center design limited the generalizability of the findings. Additionally, molecular analysis was restricted to the blaOXA-23 gene, and other resistance determinants such as blaOXA-51, blaNDM, and blaVIM were not screened. Whole genome sequencing could provide a more comprehensive understanding of the genetic environment, plasmid mediated resistance, and transmission dynamics of these isolates. Nonetheless, this study contributed important baseline data on the resistance landscape of *A. baumannii* in Lahore and highlighted the molecular mechanisms driving carbapenem resistance in Pakistan.

Conclusion

This study revealed a high prevalence of multidrug and carbapenem resistant *Acinetobacter baumannii* (*A. baumannii*) in the surgical intensive care unit of a tertiary hospital in Lahore, with the blaOXA-23 gene emerging as a major contributor to resistance, particularly among biofilm forming isolates. The identification of both regionally linked and unique Pakistani variants suggested ongoing local evolution and dissemination of resistant clones. These findings have significant implications for human healthcare, emphasizing the urgent need for robust antimicrobial stewardship, strict infection prevention protocols, and routine molecular surveillance to monitor resistance trends. Strengthening diagnostic capacity and exploring alternative or combination therapies remain crucial to mitigating treatment failures and reducing the clinical and economic burden associated with *A. baumannii* infections in hospital settings.

Authors' Contributions

ICMJE authorship criteria	**Detailed contributions**	**Authors**
Substantial Contributions	Conception or Design of the work	1,2
	Data acquisition	3,4
	Data analysis or interpretation	1,3,4
Drafting or Reviewing	Draft the work	2
	Review critically	2,3
Final approval	Final approval of the version to be published.	1,2,3,4
Accountable	Agreement to be accountable for all aspects of the work.	1,2,3,4

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