A Review of the Prevalence of Acinetobacter That Contains the blaNDM-1 Gene

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of

B.Sc. in Microbiology

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Declaration

It is hereby declared that

- The thesis submitted is my/our original work while completing a degree at Brac University.
- The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
- The thesis does not contain material that has been accepted or submitted for any other degree or diploma at a university or other institution.
- We have acknowledged all main sources of help.

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Approval

The thesis titled "A Review of the Prevalence of *Acinetobacter* That Contains the *bla*$_{\text{NDM-1}}$ Gene" submitted by

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Ethics Statement

Hereby, We, Ashikan Rabbi and Atoshi Debnath Tooli, consciously assure that for the manuscript "A Review of the Prevalence of *Acinetobacter* That Contains the *bla*$_{\text{NDM-1}}$ Gene" the following is fulfilled:

1) This material is the authors' original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis truthfully and completely.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed (correct citation). Copying of text must be indicated by using quotation marks and giving proper references.

7) All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content.

Abstract

The *bla*$_{\text{NDM-1}}$ gene is responsible for multidrug resistance in a wide variety of organisms including *Acinetobacter* which are responsible for opportunistic infections in immunocompromised patients. The gene is primarily found in Asia; however it has been detected in various parts of the world. We performed a review of the prevalence of the *bla*$_{\text{NDM-1}}$ gene in members of the *Acinetobacter species* throughout the world. We performed a literature review in PubMed using "ndm-1" and "*Acinetobacter*". Eighty eight articles were included in the study and the results showed that *bla*$_{\text{NDM-1}}$ positive *Acinetobacter* are most prevalent in Asia (64.7 %) and Africa (33 %), they are primarily found in clinical samples (80.4 %). The studies were mostly conducted in South East Asia, China, Middle East and North Africa and the proportion of *bla*$_{\text{NDM-1}}$ positive *Acinetobacter* was usually higher in countries from these regions.

Keywords: *Acinetobacter*; NDM-1, New Delhi metallo-beta-lactamase

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We would like to thank Professor Fahim Kabir Monjurul Haque, PhD, Assistant Professor, Department of Mathematics and Natural Sciences and Mahboob Hossain, PhD, Professor, Department of Mathematics and Natural Sciences for guiding us through our thesis and helping us in every possible way.

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List of Acronyms

Bla	Beta-lactamase
NDM	New Delhi metallo-beta-lactamase
K. pneumoniae	*Klebsiella pneumoniae*
E. coli	*Escherichia coli*
A. baumannii	*Acinetobacter baumannii*
CRAB	Carbapenem Resistant *Acinetobacter baumannii*
WHO	World Health Organization
Acinetobacter spp.	*Acinetobacter species*
BD	Bangladesh
KSA	Kingdom of Saudi Arabia
S. Korea	South Korea
PCR	Polymerase chain reaction
WGS	Whole genome sequencing
PFGE	Pulse field gel electrophoresis
MLST	Multi – Locus Sequence Typing
ICU	Intensive care unit
env.	Environment

Introduction

The *bla*$_{\text{NDM-1}}$ gene was first detected in patients in Sweden who had previously traveled to New Delhi, India back in 2009 [1]. Ever since, the gene has spread to almost every part of the world including countries in Europe, Africa and the Americas [2]. The spread of the gene is of major concern as it is a carbapenemase gene which can give rise to resistance against a wide variety of antibiotics [3]. Carbapenems are effective against resistant bacteria that can produce β – lactamase. However, metallo - β – lactamases (MBLs) can break down Carbapenems [4]. Bacteria containing the *bla*$_{\text{NDM-1}}$ gene can hydrolyze all β – lactam antibiotics except aztreonam [5]. The *bla*$_{\text{NDM-1}}$ gene poses a major threat due to its multidrug resistant nature and its ability to spread via plasmid conjugation [6]. Therefore, we have limited options when it comes to treatment of infections caused by *bla*$_{\text{NDM-1}}$ positive organisms. The *bla*$_{\text{NDM-1}}$ gene can be found in a number of infectious and non-infectious bacteria such as *Klebsiella pneumoniae, Escherichia coli* and *Acinetobacter baumannii* [7]. It has been isolated from clinical samples such as urine, blood and respiratory samples. It has also been detected in environmental samples such as water samples and animal feces [16] [56].

Acinetobacter are Gram-negative saprophytic organisms that are non-fermentative facultative anaerobes. They are commonly found in soil, wastewater, vegetables and human and animal skins [8]. Members of the *Acinetobacter species* of bacteria are opportunistic pathogens that can cause infections in immunocompromised patients [9]. Despite their low virulence, multidrug resistant *Acinetobacter* infections are of major concern in hospital settings as they target patients who are already sick and due to their resistance, treatment becomes very difficult [10]. CRAB (Carbapenem-resistant *A. baumannii*) has been added to the “critical group” of bacteria by the World Health Organization (WHO) that poses the greatest threat to

human health and requires more research [10]. The *bla*$_{NDM-1}$ gene can be transferred to *Acinetobacter* from both members of the *Acinetobacter spp.* and other species [12] [13].

Due to the threat posed by multi drug resistant *Acinetobacter* infections and the multi-drug resistant nature of the *bla*$_{NDM-1}$ gene, we wanted to look into the prevalence of *bla*$_{NDM-1}$ positive *Acinetobacter* organisms. The purpose of this review was to analyze the spread of the *bla*$_{NDM-1}$ gene among *Acinetobacter* throughout the world and in different settings as these organisms can cause opportunistic infections that would be difficult to cure due to their multi drug resistant nature.

Materials and methods

Literature search

A literature search was performed for the prevalence of the *bla*$_{NDM-1}$ gene in members of the *Acinetobacter spp*. The search was performed through the PubMed electronic database between March 2021 and November 2021. The keywords used in the search process were: ndm-1, and, *Acinetobacter*.

Inclusion and exclusion criteria

Articles were selected for review based on title, followed by a read through the abstract and finally if it appeared to be relevant, we went through the full article. Articles that were included had to specify the method of identification of *bla*$_{NDM-1}$ gene in organisms, the origin of the organisms and the number of *Acinetobacter* containing the *bla*$_{NDM-1}$ gene. Conversely the exclusion criteria were articles that did not specify the exact source of the samples, not mentioning the number of samples tested or the number of samples that had *bla*$_{NDM-1}$ positive *Acinetobacter*. Systematic reviews, duplicates of already reviewed articles and any article that did not have a full text available were also excluded.

Data extraction and definition

The information obtained from each of the chosen articles include: author's name, period of study, year of publication, type of sample, number of sample, source of sample, method of detection of *bla*$_{\text{NDM-1}}$ gene, number of *Acinetobacter* and number of *bla*$_{\text{NDM-1}}$ positive *Acinetobacter*. In some cases not all of the information could be obtained, however for all the articles source of the sample, method of detection of the *bla*$_{\text{NDM-1}}$ gene and the number of *bla*$_{\text{NDM-1}}$ positive *Acinetobacter* was mentioned. In some cases the study worked with resistant organisms only and that has been noted in the data. A condensed version of the chosen articles along with some of the data collected is shown in Table 1. Some papers are listed twice as they worked with two or more different types of samples. The information for each type of sample is listed separately.

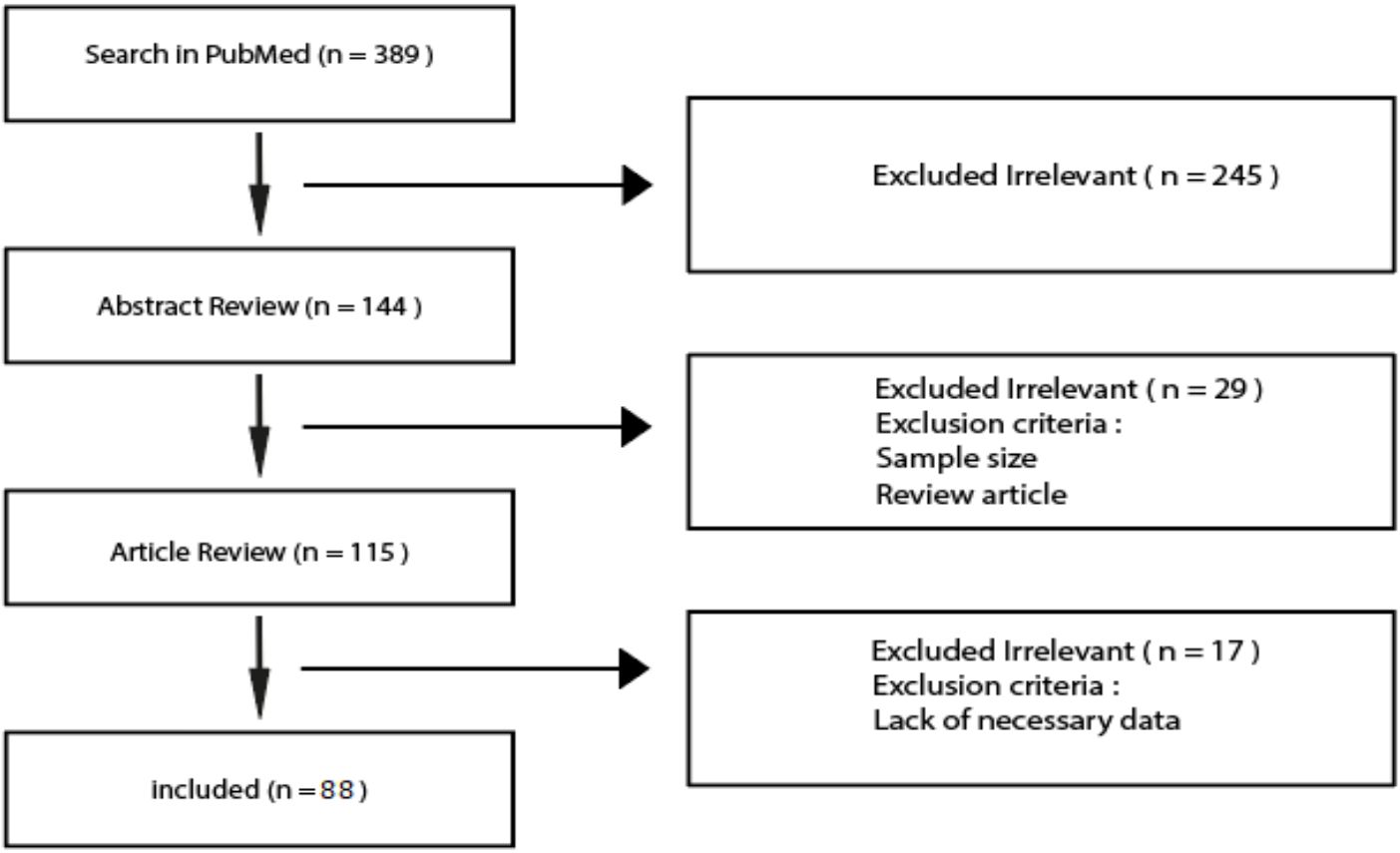

Figure 1: Flow chart of article selection for the review

First author	Period of study	Year of publication	Country of origin	Method of identification	Number of *Acinetobacter*	Number of *bla*$_{NDM-1}$ *Acinetobacter*	Type of sample
Chen Y. [14]	2009 - 2010	2011	China	PCR	2109	4	Clinical sample
Islam M. A. [7]	2010 - 2010	2012	BD	PCR	18	3	Clinical sample
Mataseje L. F. [15]	2009 - 2010	2012	Canada	PCR	9	0	Enterobacteriaceae
Murali S. [18]	2011 - 2011	2012	India	PCR	1	1	Donor Eye
Wang Y. [16]	2010 - 2010	2012	China	PCR	1	1	Animal swabs
Yang J. [17]	2008 - 2009	2012	China	PCR	3114	27	Clinical sample
Farzana R. [20]	2010 - 2011	2013	BD	PCR	15	4	Clinical sample
Hasan B. [24]	2010 - 2011	2013	Pakistan	PCR	90	1	Clinical sample
Mesli E. [19]	2008 - 2012	2013	Algeria	PCR	113	5	Clinical sample
Revathi G. [23]	2009 - 2010	2013	Kenya & Rwanda	PCR	16	1	Clinical Sample
Yanik K. [25]	-	2013	Turkey	PCR	132	0	Clinical sample
Zhang C. [22]	2010 - 2010	2013	China	PCR	42	13	Water sample
Zhang R. [21]	2010 - 2013	2013	China	PCR	1067	7	ICU env.
Ageevets V. A. [32]	2011 - 2013	2014	Russia	PCR	-	1	Clinical sample
Bakour S. [26]	2011 - 2013	2014	Algeria	PCR	47	11	Clinical sample
Cicek A. C. [35]	2011 - 2012	2014	Turkey	PCR	101	0	Clinical sample
Jones R. N. [29]	2011	2014	Europe	PCR	472	0	Clinical sample
Kulkova N. [33]	2011 - 2012	2014	Slovakia	PCR	9	0	Blood culture
Lauderdale T. L. [34]	2010	2014	Taiwan	PCR	408	1	Clinical sample
Pasteran F. [31]	2012	2014	Paraguay	PCR	2	2	Clinical sample
Peirano G. [27]	2010 - 2013	2014	Canada	PCR	1	0	Clinical sample
Rafei R. [30]	2012	2014	Lebanon	PCR	4	4	Clinical sample
Zheng F. [28]	2010 - 2010	2014	China	PCR	169	2	Clinical sample
Ahmed M. A. [38]	2012 - 2013	2015	Egypt	PCR	150	59	Clinical sample
Memish Z. A. [40]	2012 - 2012	2015	KSA	PCR	79	1	Clinical sample
Novovic K. [43]	2012 - 2014	2015	Serbia	PCR	28	0	Clinical sample
Quiñones D [37].	2010 - 2012	2015	Cuba	PCR	500	1	Clinical sample
Rafei R. [41]	2011 - 2013	2015	Lebanon	PCR	116	5	Clinical sample
Shrestha S. [42]	2013 - 2014	2015	Nepal	MLST	246	51	Clinical sample
Sung J. Y. [39]	2006 - 2013	2015	S. Korea	PCR	21	2	Clinical sample
Tran H. H. [44]	2010 - 2012	2015	Vietnam	PCR	31	3	Hospital env. sample
Zenati K. [36]	2011 - 2013	2015	Algeria	PCR	67	32	Hospital env. sample
Adler A. [48]	2014 - 2015	2016	Israel	PCR	313	16	Clinical sample
Bouguenoun W. [46]	2014 - 2014	2016	Algeria	PCR	3	2	Clinical sample
Bouguenoun W. [46]	2014 - 2014	2016	Algeria	PCR	6	5	Hospital env. sample
Cetinkol Y. [51]	-	2016	Turkey	PCR	50	0	Clinical sample
Kateete D. P. [52]	2007 - 2009	2016	Uganda	PCR	29	0	Clinical sample
Kateete D. P. [52]	2007 - 2009	2016	Uganda	PCR	11	0	Hospital env. sample
Mathlouthi N. [49]	2015 - 2015	2016	Libya	PCR	36	8	Clinical sample
Ramoul A. [47]	2010 - 2013	2016	Algeria	PCR	43	7	Clinical sample
Timofte D. [50]	2014 - 2015	2016	Romania	PCR	-	0	Clinical sample
Tran D. N. 45]	2010 – 2014	2016	Vietnam	PCR	582	23	Clinical sample
El-Mahdy T. S. [59]	2014 - 2014	2017	KSA	PCR	10	3	Clinical sample
Gomaa F. A. M. [57]	2014 - 2015	2017	Egypt	PCR	56	13	Clinical sample
Hammami S. [64]	2014 - 2014	2017	Tunisia	PCR	5	0	Clinical sample
Hasan M. J. [55]	2013 - 2013	2017	BD	PCR	22	2	Clinical sample
Islam M. A. [56]	2012 - 2012	2017	BD	PCR	-	13	Water sample

Joshi P. R. [61]	2014 - 2015	2017	Nepal	PCR	44	6	Clinical sample
Manohar P. [58]	2014 - 2015	2017	India	PCR	5	0	Clinical sample
Mellouk F. Z. [54]	2013 - 2015	2017	Algeria	PCR	7	2	Clinical sample
Morakchi H. [100]	2013 - 2013	2017	France - Algeria	PCR	14	0	Pigeon stool
Pirii L. E. [62]	2015	2017	Romania	WGS	1	0	Burn patient
Romero J. L. [63]	2013 -2014	2017	Spain	PCR	4	0	Sea food
Uwingabiye J. [60]	2015 - 2015	2017	Morocco	PCR	47	9	Clinical sample
Uwingabiye J. [60]	2015 - 2015	2017	Morocco	PCR	36	18	Hospital env. Sample
Yagoubat M. [53]	2014 - 2015	2017	Algeria	PCR	-	0	Clinical sample
Yagoubat M. [53]	2014 - 2015	2017	Algeria	PCR	8	5	Hospital env. sample
Agoba E. E. [71]	2015 - 2015	2018	South Africa	PCR	24	1	Clinical sample
Banerjee T. [68]	2012 - 2016	2018	India	PCR	100	34	ICU env.
Cheikh H. B. [74]	2013 - 2016	2018	Tunisia	PCR	101	1	Clinical sample
Dziri O. [73]	2015 - 2016	2018	Tunisia	PCR	3	0	Clinical sample
Faccone D. [65]	2013 - 2015	2018	Argentina	PCR	10	1	Clinical sample
Gentilini F. [70]	2014 - 2015	2018	Italy	PCR	6	1	Pets
Jaidane N. [75]	2013 - 2015	2018	Tunisia	PCR	246	7	Clinical sample
Jain M. [66]	2016 - 2017	2018	India	PCR	28	3	Clinical sample
Jain M. [66]	2016 - 2017	2018	India	PCR	8	0	ICU env.
Kuntaman K. [69]	2015 - 2016	2018	Indonesia - Japan	PCR	75	6	Clinical sample
Leungtongkam U. [72]	2013 - 2015	2018	Thailand	PCR	339	31	Clinical sample
Maamar E. [76]	2014 - 2015	2018	Tunisia	PCR	13	2	Clinical sample
Rahman M. [67]	2012 - 2012	2018	India	PCR	106	20	Clinical sample
Abouelfetouh A. [78]	2010 and 2015	2019	Egypt	PCR	74	9	Clinical sample
Al - Hamad A. [81]	2014	2019	KSA	PCR	21	1	Clinical sample
Al - Hamad A. [81]	2014	2019	KSA	PCR	74	1	Hospital env. Sample
Anane Y. A. [84]	2016 - 2017	2019	South Africa	PCR	52	0	Fish body and water
Anane Y. A. [84]	2016 - 2017	2019	South Africa	PCR	48	0	Slaughterhouse env.
Gomez L. G. [86]	2016	2019	Venezuela	Plasmid profile, PFGE, MLST	8	3	Clinical sample
Khalid S. [79]	2017 - 2017	2019	India	PCR	-	4	Clinical sample
Kumar S. [80]	2013 - 2016	2019	India	PCR	97	25	Clinical sample
Lee Y. [85]	2018	2019	Taiwan	PCR	188	0	Clinical sample
Qamar M. U. [83]	2015 - 2016	2019	Pakistan	PCR	29	4	Clinical sample
Rakhi N. N. [77]	2016	2019	BD	PCR	4	2	Clinical sample
Shah M. W. [82]	2015 - 2016	2019	KSA	PCR	135	2	Clinical sample
Anane Y. A. [95]	2016 - 2017	2020	South Africa	PCR	100	2	Clinical sample
Benamrouche N. [87]	2012 - 2016	2020	Algeria	PCR	92	5	Clinical sample
Kalasseril S. G. [90]	2017 - 2018	2020	India	PCR	2	0	Hospital env. Sample
Kongthai P. [96]	2013 - 2015	2020	Thailand	PCR	339	1	Clinical sample
Lukovic B. [94]	2018 - 2018	2020	Serbia	PCR	280	7	Clinical sample
Manandhar S. [93]	2012 - 2018	2020	Nepal	PCR	383	79	Clinical sample
Moubareck C. A. [97]	2015 - 2016	2020	UAE	PCR	341	1	Clinical sample

Rao M. [91]	2011 - 2016	2020	Malaysia	WGS	13	3	Clinical sample
Sanou S. [88]	2016 - 2016	2020	Burkina Faso	PCR	4	1	Clinical sample
Sharma M. [89]	2013 - 2014	2020	India	PCR	150	28	Clinical sample
Tada T. [92]	2015 - 2018	2020	Myanmar	WGS	45	5	Clinical sample
Monnheimer M. [98]	2017 - 2018	2021	Ghana	PCR	45	19	Clinical sample
Soliman A. M. [99]	2014 - 2015	2021	Japan	PCR	1	0	Vegetable

Table 1: List of selected articles

BD, Bangladesh; S. Korea, South Korea; KSA, Kingdom of Saudi Arabia; UAE, United Arab Emirates; env., environment.

Results

Prevalence by continent

When looking at the number of NDM-1 positive organisms found by continent, out of a total of 684 NDM-1 positive organisms, 441 were from Asia, 225 were from Africa, 9 from Europe, 6 from South America and lastly 1 from North America. The numbers were obtained from 87 papers (one paper was excluded as the location was not limited to one continent) of which 47 were based in Asia (Turkey was counted under Asia), 25 were from Africa, 9 were from Europe, 3 were from North America and 3 were from South America. Figure 2 shows a pie chart for the distribution of the detected organisms among the 5 continents and figure 3 is a line graph to show the relation between the numbers of papers compared to the number of organisms detected.

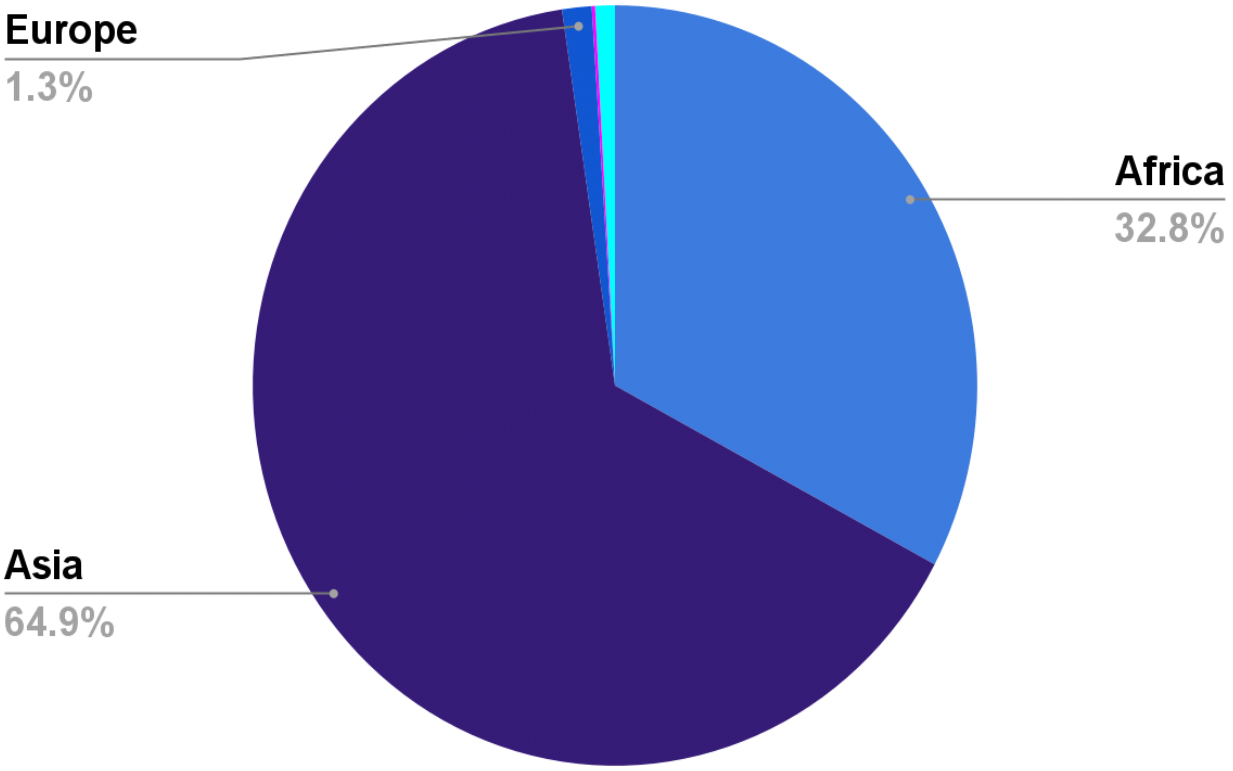

Figure 2: Pie chart of blaNDM-1 positive organisms by continent

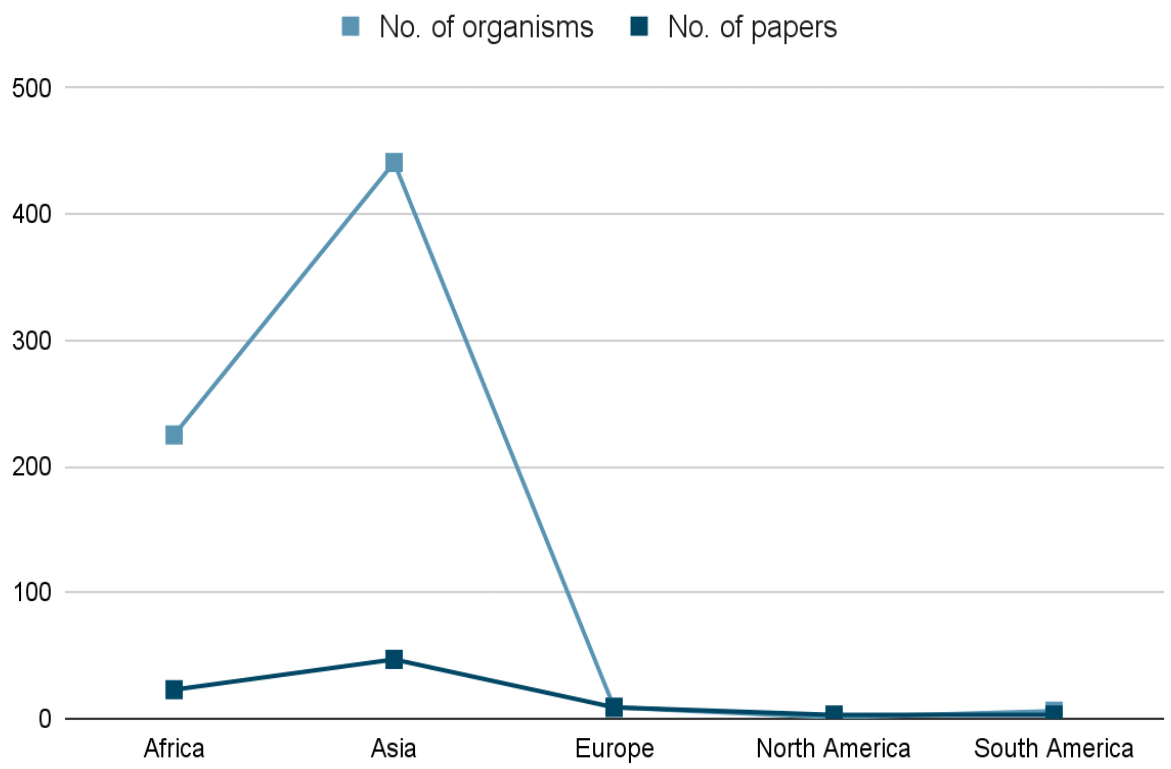

Figure 3: Line graph to compare the number of blaNDM-1 positive organisms with the number of papers found for each continent

Prevalence by country

We found data for 37 different countries after excluding papers where multiple countries were involved. The country with the greatest number of articles was India with 9 separate papers followed by Algeria which had 8 papers. However, the highest number of NDM-1 positive *Acinetobacter* was found in Nepal at 136 followed by India with 115. Figure 4 shows the total number of NDM-1 positive organisms found for each country based on the data reviewed. Any country with ten or less organisms detected falls under the "Others" column. This includes 26 different countries. Among them Tunisia had the most with 10 detected organisms, followed by Lebanon and Libya both with 9 detected organisms each. Six of the twenty-six countries had zero NDM-1 positive *Acinetobacter* which were Canada, Japan, Slovakia, Spain, Turkey and Uganda. A scatter plot (figure 5) is also included to show the

relation between number of organisms detected and the number of papers for each country. Table 2 shows the full list for all the countries.

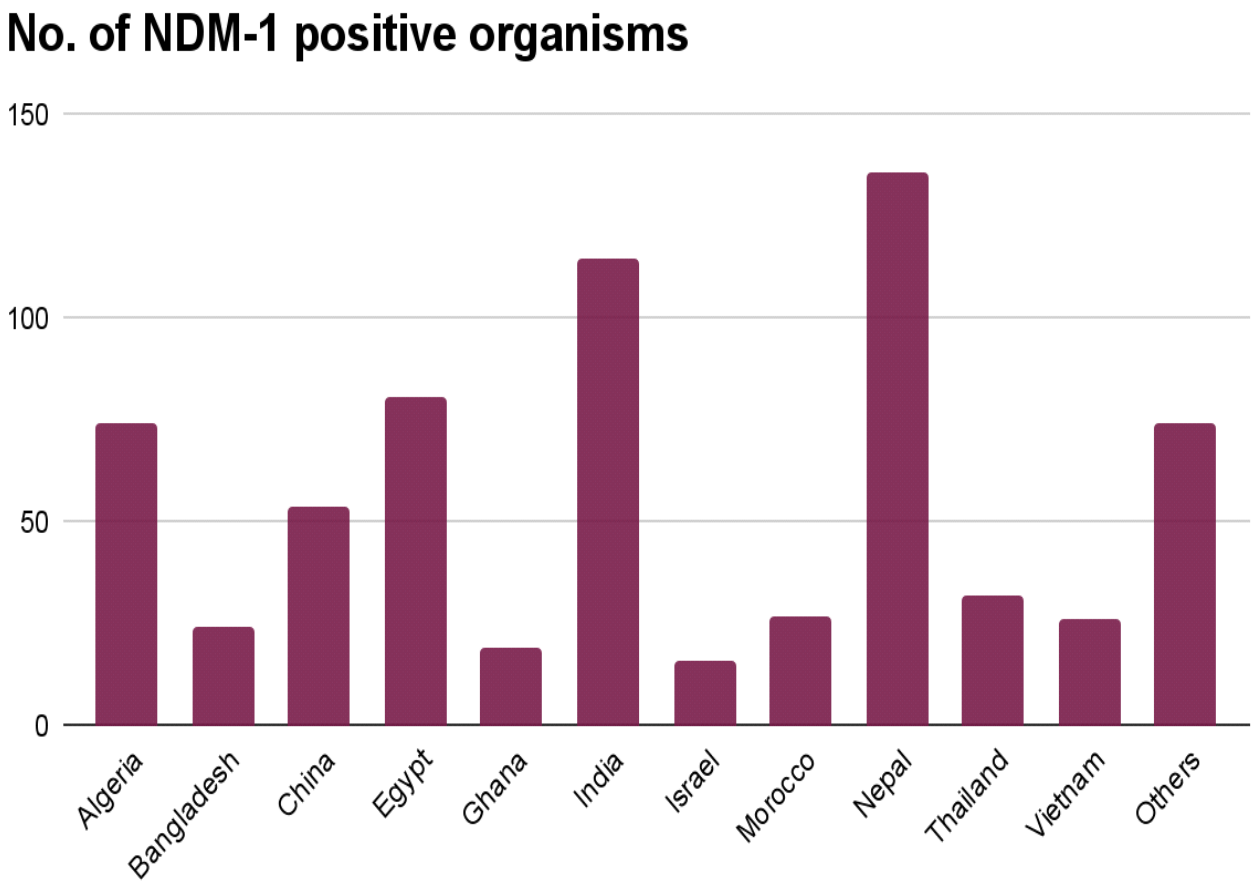

Figure 4: Chart showing number of blaNDM-1 positive organisms by country

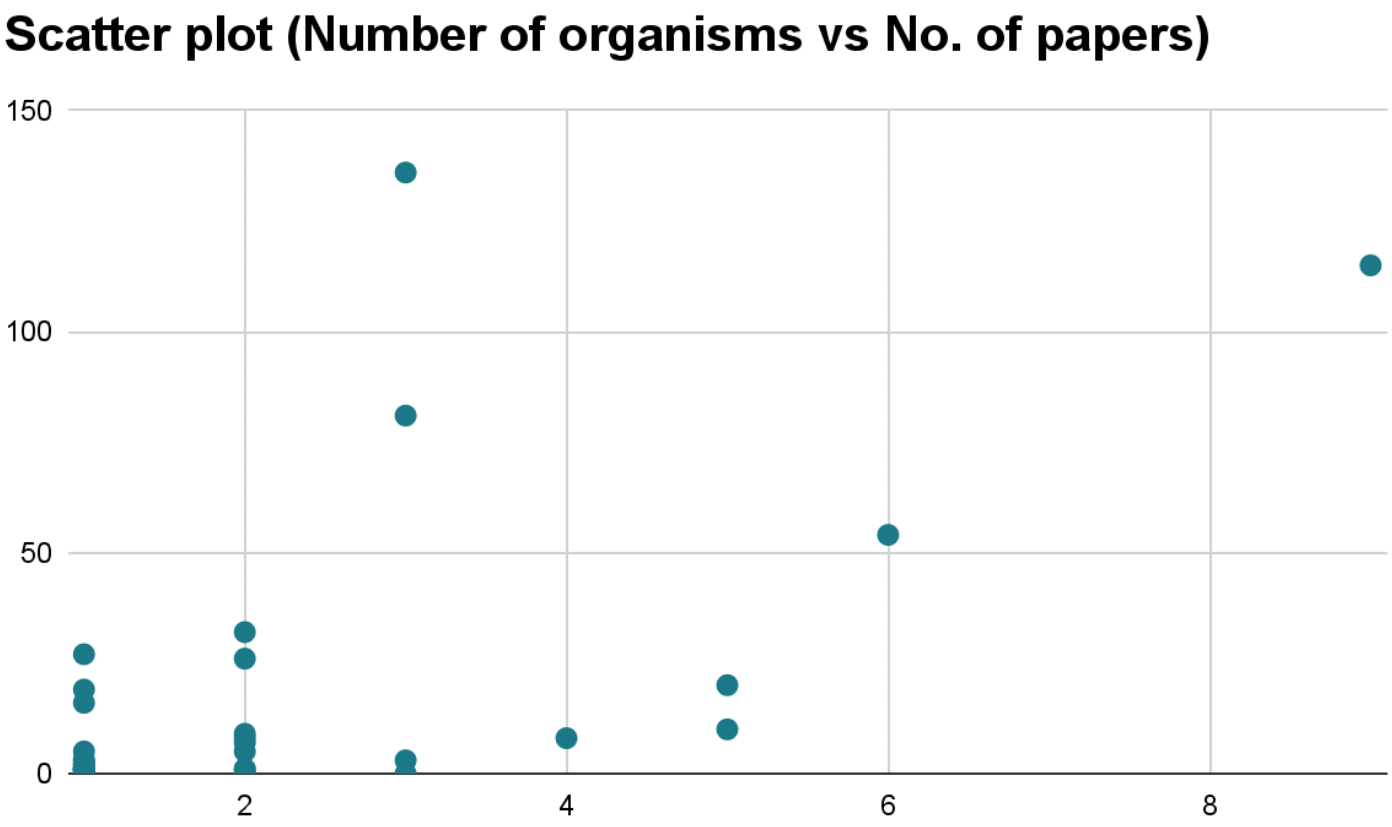

Figure 5: Scatter plot of blaNDM-1 positive organisms by country against the number of articles reviewed for each country

Country of origin	No. of organisms	Country of origin	No. of organisms	Country of origin	No. of organisms
Algeria	74	South Korea	2	Slovakia	0
Argentina	1	KSA	8	South Africa	3
Bangladesh	24	Lebanon	9	Spain	0
Burkina Faso	1	Libya	8	Taiwan	1
Canada	0	Malaysia	3	Thailand	32
China	54	Morocco	27	Tunisia	10
Cuba	1	Myanmar	5	Turkey	0
Egypt	81	Nepal	136	UAE	1
Ghana	19	Pakistan	5	Uganda	0
India	115	Paraguay	2	Venezuela	3
Israel	16	Romania	1	Vietnam	26
Italy	1	Russia	1	**Total**	**678**
Japan	0	Serbia	7		

Table 2: Number of blaNDM-1 positive organisms by country

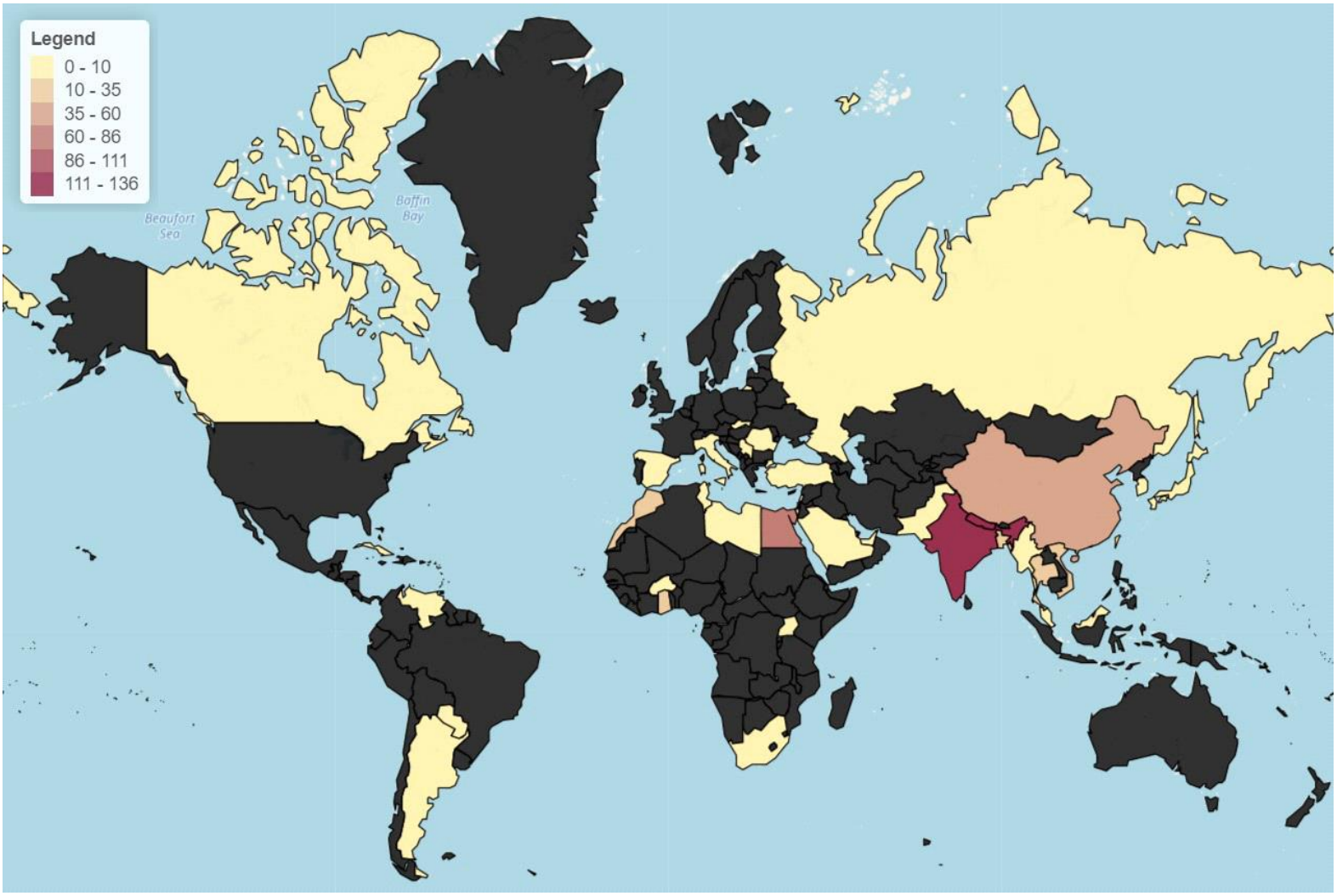

Figure 6: Heat map of Number of positive Acinetobacter by country

Prevalence by source

The source of the organism can be broadly classified into three categories. These are clinical specimens, samples from hospital environment and lastly other samples which include samples from water (excluding water from hospital sources), food, plants and animals. The pie chart in figure 6 shows the distribution of 683 NDM-1 positive organisms based off the source of the organism. The data for the number of papers for each source is also given, however some papers which tested samples from different sources have been counted for all types of samples they tested. For example, papers that tested both a hospital environment and clinical samples were counted for both hospital environment and clinical samples. A line graph of the number of NDM-1 positive organisms detected to the number of papers is presented in figure 7.

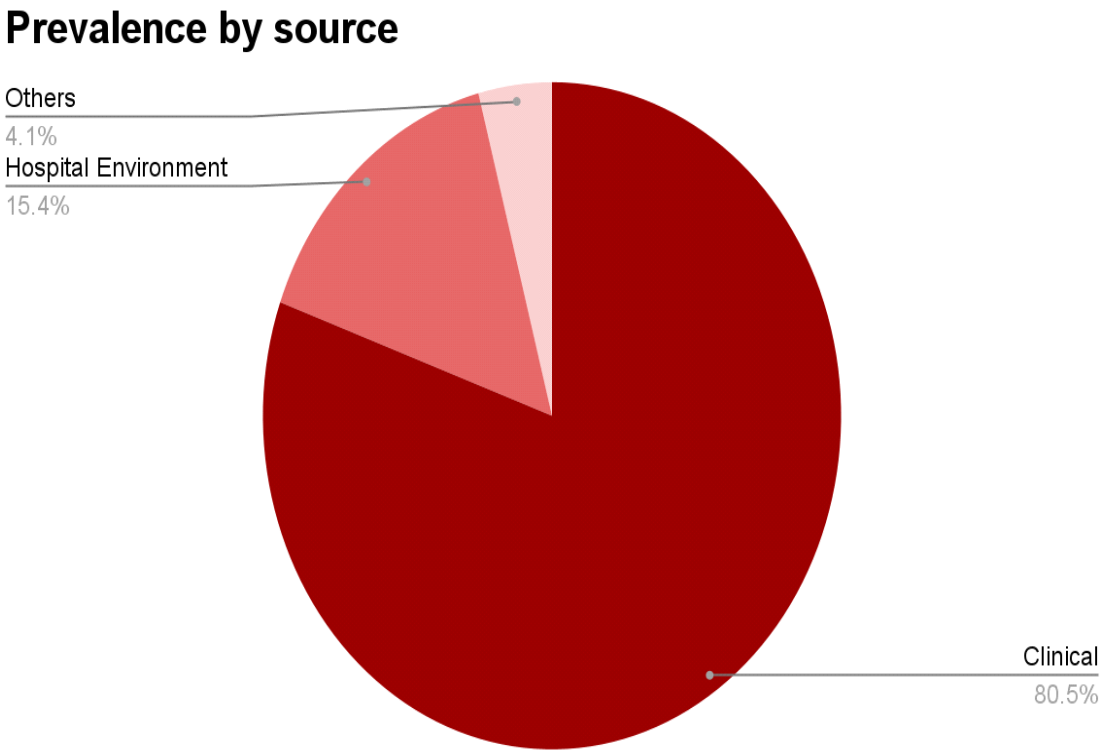

Figure 7: Pie chart of blaNDM-1 positive organisms by source of sample

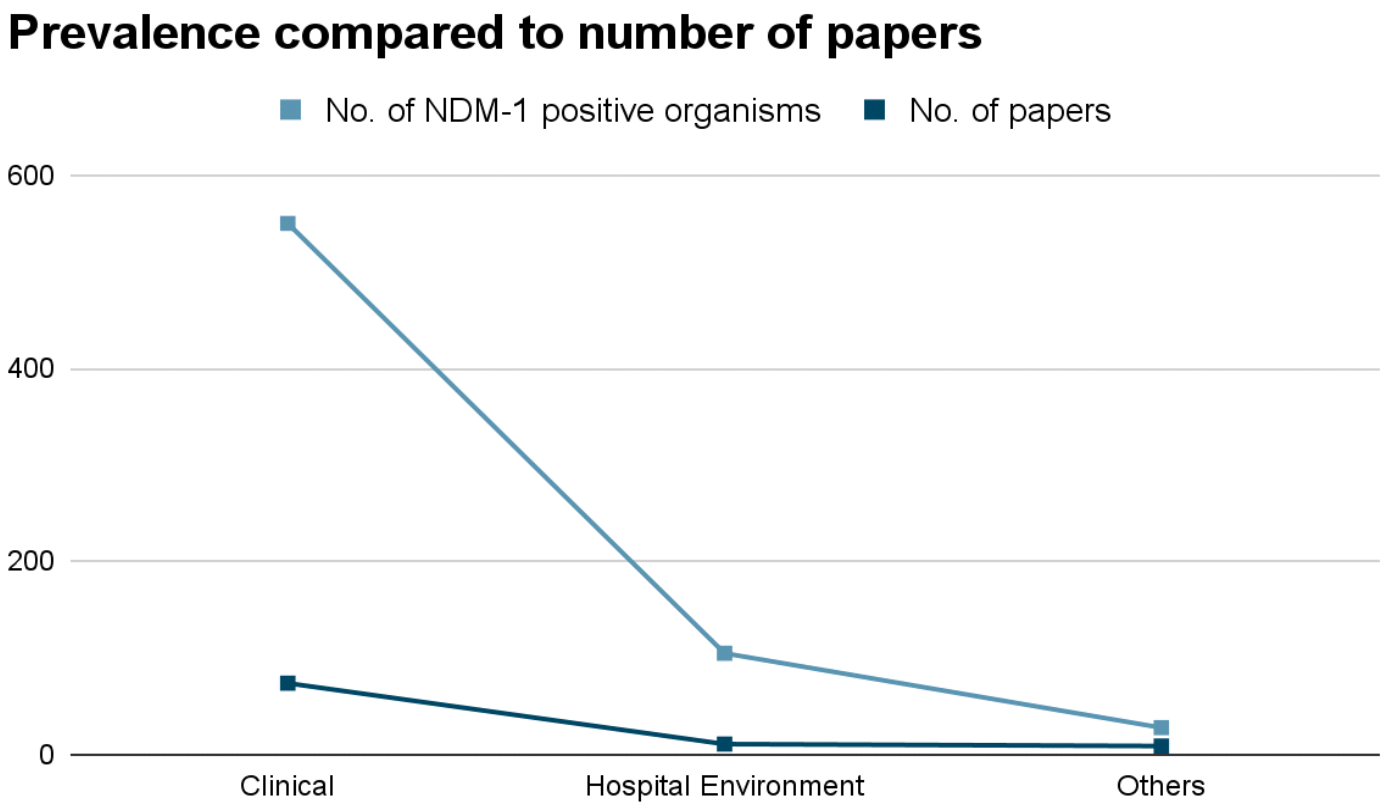

Figure 8: Line graph of the number of blaNDM-1 positive organisms against the number of papers reviewed based on the source of the sample

Prevalence by resistant *Acinetobacter*

It is difficult to find the percentage of *Acinetobacter* that are NDM-1 positive from the data since the total number of *Acinetobacter* detected is not mentioned for some studies. For the studies where the number of *Acinetobacter* detected was mentioned, some only worked with resistant organisms while other did not so we cannot count them under the same category. We therefore broke down the data into the number of resistant *Acinetobacter* that turned out to be NDM-1 positive. Resistant organisms mainly constitute *Acinetobacter* that were carbapenem resistant or multidrug resistant. The chart below (figure 8) shows the log of the number of resistant *Acinetobacter* that are NDM-1 positive for each country alongside the log of the number of resistant *Acinetobacter* tested. We used the logarithmic values as it was easier to visualize and compare. This is because the sample size for each country varies greatly. This

means it would not be accurate to compare the proportion of resistant organisms with the proportion of NMD-1 positive organisms in the chart, but we can at least get an idea of how the values vary from country to country. Values for Lebanon, Paraguay and Venezuela were excluded as the numbers of resistant organisms detected in these countries were less than 10 which were not enough to provide an accurate representation. The chart also does not show the countries where resistant *Acinetobacter* were found but none of the organisms were NDM-1 positive. This includes Japan and Romania. The highest percentage of NDM-1 positive organisms among resistant *Acinetobacter* was found in Algeria (35.94%) followed by Bangladesh (34.78%) and then Morocco (32.53%). List of the percentage for each country is given in table 3.

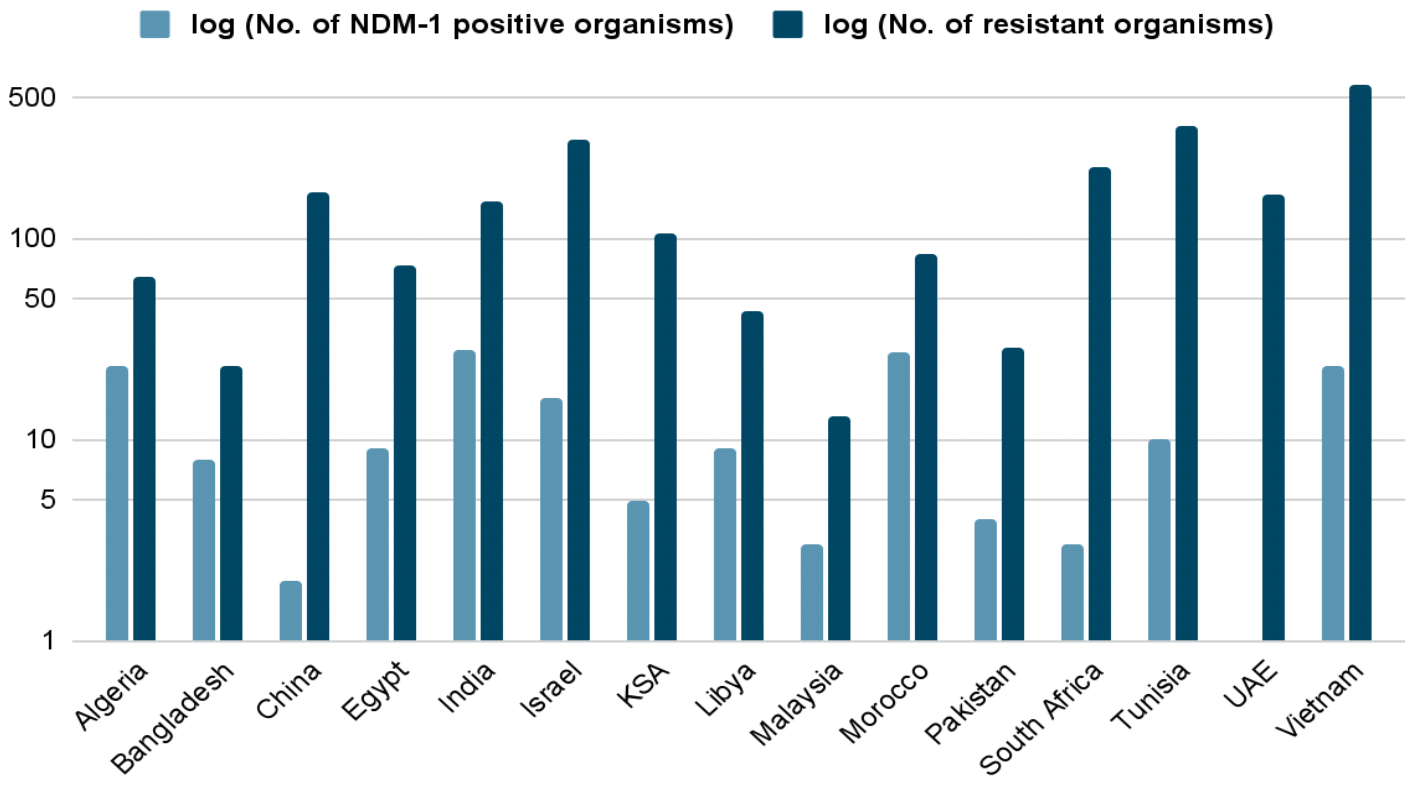

Figure 9: Chart showing the log of the number of resistant organisms detected alongside the number of NDM-1 positive organisms among them by country

Country of origin	No. of resistant *Acinetobacter* detected	Percentage of resistant *Acinetobacter* that are NDM-1 positive (%)
Algeria	64	35.94
Bangladesh	23	34.78
China	169	1.18
Egypt	74	12.16
India	152	18.42
Israel	313	5.11
KSA	105	4.76
Libya	44	20.45
Malaysia	13	23.08
Morocco	83	32.53
Pakistan	29	13.79
South Africa	224	1.34
Tunisia	360	2.78
UAE	167	0.6
Vietnam	582	3.95

Table 3: Percentage of resistant Acinetobacter that are NDM-1 positive

If we consider the number of NDM-1 positive organisms compared to the number of resistant organisms with respect to the source we get the results shown in figure 9. We used the log of the values once again as it is difficult to compare them directly due to the large size of clinical samples. Therefore the proportion is not as significant as it appears. If we break down the values by percentage, out of 2166 resistant clinical isolates 6.65% (144) were NDM-1 positive. Out of 120 resistant *Acinetobacter* samples from hospital environments, 20% (24) were NDM-1 positive. Lastly, out of 108 resistant environmental samples, 0.93% (1) was NDM-1 positive.

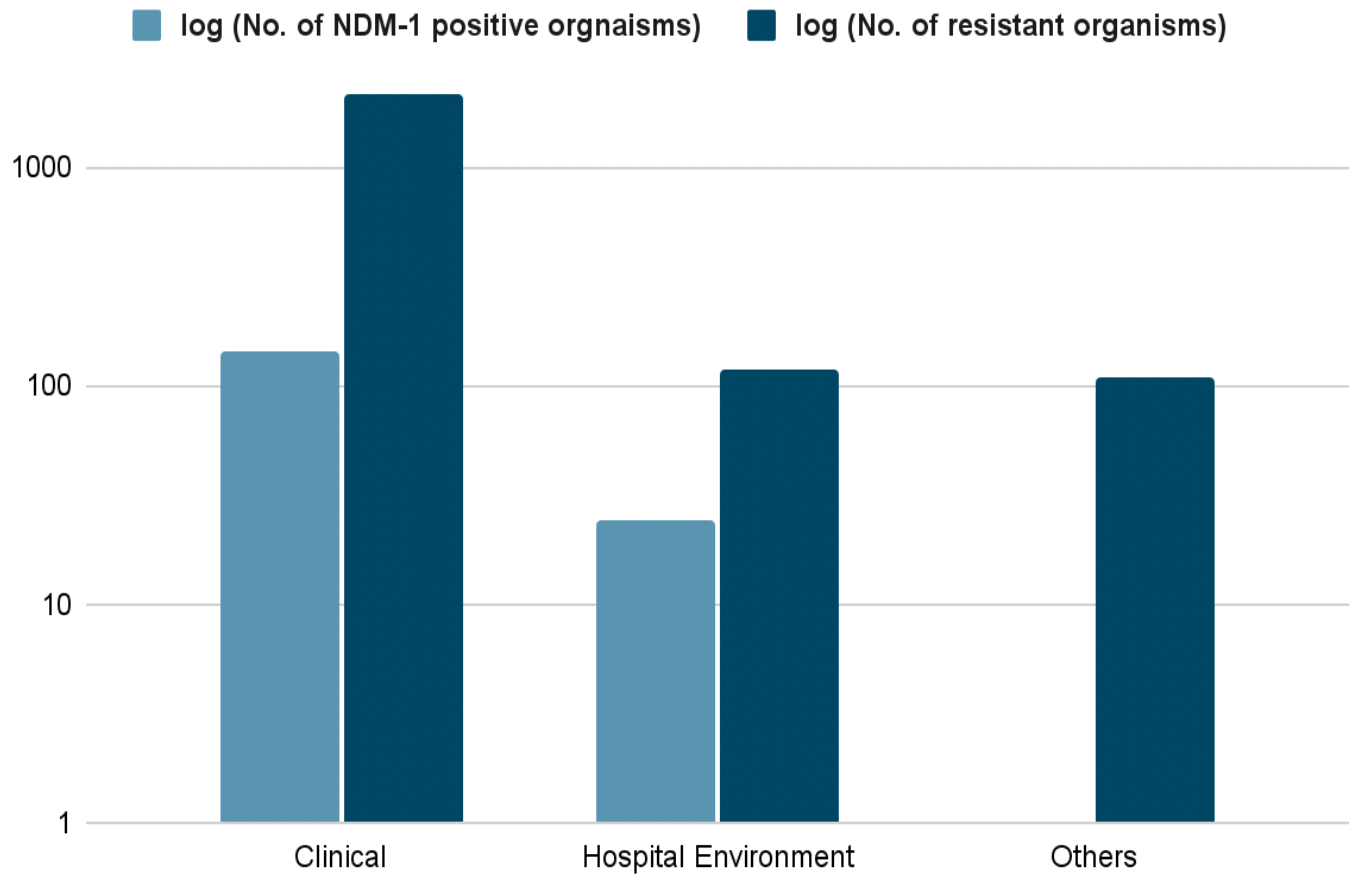

Figure 10: Chart showing the log of the number of resistant organisms detected alongside the number of NDM-1 positive organisms among them by source

Prevalence by year

Prevalence by year is difficult to represent accurately as most of the studies took place over two or more years. Outside of studies that were only conducted in a single year, we also counted studies where the number of months in a particular year was double that of any other year for the study. For example, if a study took place from December 2014 till August 2015, we counted the study for 2015 as the number of months in 2015 was more than double that spent in 2014 for this study. Figure 10 shows a chart of this data alongside the number of papers from which the data was collected.

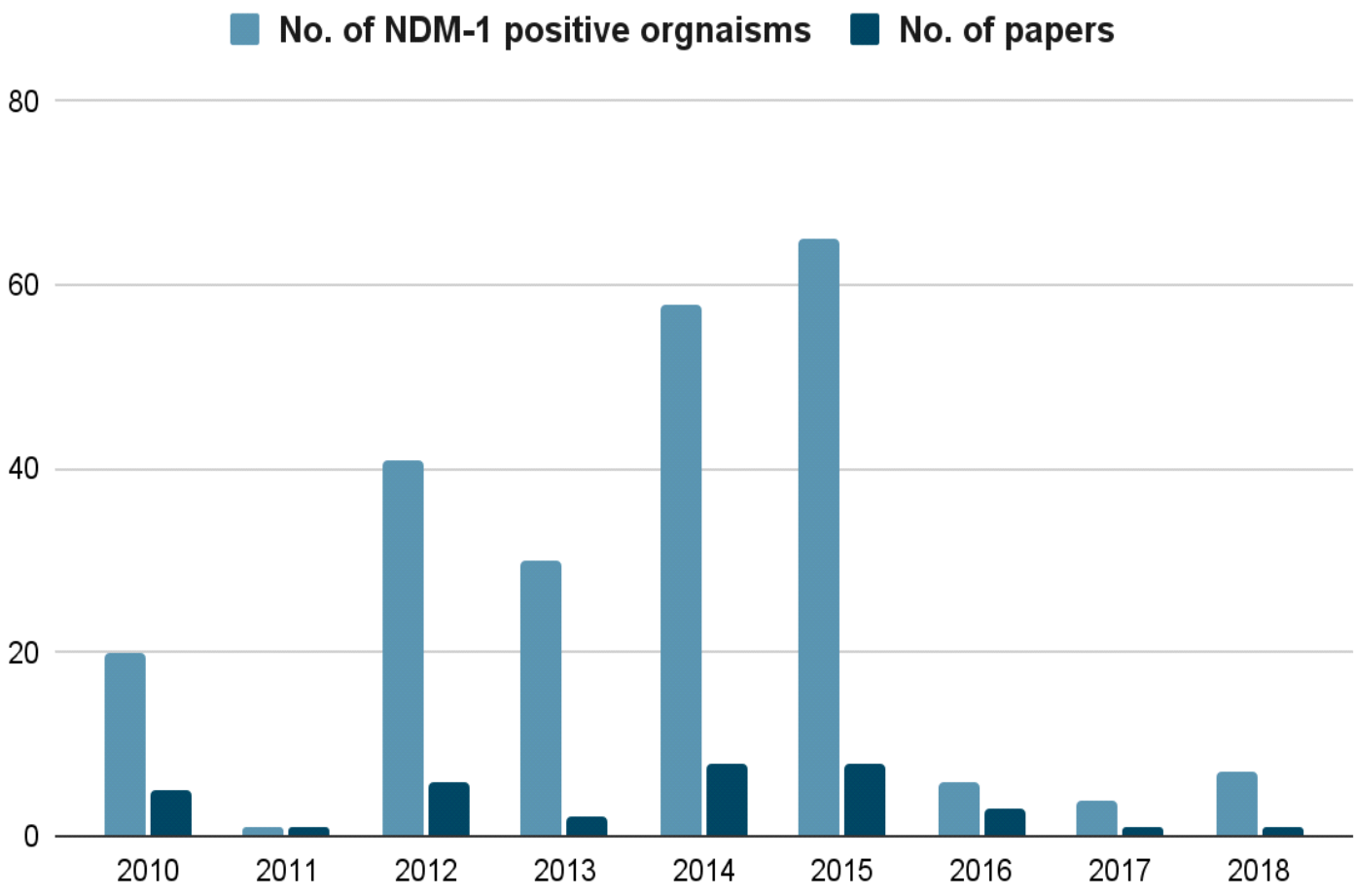

Figure 11: Chart showing the number of NDM-1 positive organisms detected by year alongside the number of papers counted for each year

Discussion

Based on the articles reviewed, members of the *Acinetobacter spp.* that are NDM-1 positive are most commonly found in Asia followed by Africa. From our results more than half of the identified NDM-1 positive *Acinetobacter* were from Asia and about one third were from Africa. The rest of the world constitutes less than three percent of the results. By country, the highest numbers of NDM-1 positive *Acinetobacter* were from Nepal and then India. The data was compared with the number of articles found for each country. While more articles did tend towards more organisms, there was no clear relation between the two factors. However, this does not imply that Nepal has the largest prevalence of NDM-1 positive *Acinetobacter* as we couldn't take other factors into account such as number of organisms tested. It is difficult to consider the number of *Acinetobacter* tested as some studies only worked with resistant *Acinetobacter* while some others did not mention whether the *Acinetobacter* were resistant or not. Thus the data should be considered merely as an indicator for where the NDM-1 gene may be more prevalent.

In terms of source, majority of the organisms were from clinical samples with less than twenty percent of the detected organisms being from other sources. Less than five percent of the organisms were not from a hospital. When comparing this data with the number of papers found for each source there does appear to be a positive correlation between the number of papers for a particular source and the number of NDM-1 positive *Acinetobacter* found for the that particular source. Since very few samples were taken from non-hospital settings, it is hard to determine the proportion of organisms outside of hospital settings that may be NDM-1 positive. However, based off the sample size that was available it does appear the proportion of *Acinetobacter* that are NDM-1 positive is higher in hospitals compared to other sources.

When considering the proportion of resistant *Acinetobacter* that were NDM-1 positive, we find that the percentage is higher for countries in South East Asia and Northern Africa. In most cases the percentage was lower when the number of tested samples were greater, however this also varied with region. The highest percentages were from Algeria, Bangladesh and Morocco were around a third of the resistant organisms were also NDM-1 positive. However in all three of these countries the numbers of resistant organisms detected were less than 100. For countries were over 100 resistant organisms were tested, the percentage was around 5 percent or lower, excluding India where out of 152 resistant organisms around 18 percent were NDM-1 positive.

If we look at the percentage of resistant *Acinetobacter* that are also NDM-1 positive on the basis of setting, we find that the largest percentage was for hospital environment at twenty percent, followed by clinical specimen at around six percent. The percentage for non-hospital settings was less than one percent. However, if we take the number of organisms into account, the sample size is a lot larger for clinical specimens at over 2000 while the number of samples for both hospital environment and non-hospital settings were just over 100. It is likely that the percentage for hospital environment would be lower if we had a larger sample size.

Lastly we broke down the data in a year by year basis. This was made difficult by the fact that most studies took place over a multiyear time period. We had to exclude a lot of studies as a result. Alongside studies that took place over a single year, we also counted papers where the study period in a particular year was at least double the study period in any other year. According to the results the most NDM-1 positive organisms were detected between 2012 and 2015. The number of papers published in this time frame was also more than other years.

While there are more NDM-1 positive organisms detected in years where more papers were published, it is hard to say for certain if the two factors are co-related.

Conclusion

There needs to be more research into *Acinetobacter* with the NDM-1 gene, especially in regions around Asia and Africa where it is more prevalent. In the age of globalization where people travel far and often it is very easy for the $bla_{\text{NDM-1}}$ gene to spread. Treatment for infections caused by NDM-1 positive *Acinetobacter* is very limited and can be life threatening. Thus it is important for us to monitor the spread of this gene in various organisms in order to control its spread. While the primary source of NDM-1 positive *Acinetobacter* is clinical specimens, we cannot ignore other sources either. Even though clinical specimens are the most likely to cause opportunistic infections, environmental cases of NDM-1 positive *Acinetobacter* can contribute to the spread of the NDM-1 gene to other organisms in the same species as well as other species through plasmid conjugation.

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