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Review

A systematic review and meta-analysis on Exo-toxins prevalence in hospital acquired *Pseudomonas aeruginosa* isolates

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* (PA) is an opportunistic pathogen that produces widespread and often overwhelming infections. Among different virulence factors, toxins are important bacterial agent which increases PA pathogenesis especially in immunocompromised patients. The aim of this meta-analysis was to determine the prevalence of exotoxin production in PA isolates in the world. Also according to the importance of drug resistance in isolates with more pathogenicity this estimation was conducted in resistant isolates.

Methods: A systematic search was conducted in international database like PubMed, Scopus, Web of Science and Embase up to December 2018. Joanna Briggs Institute Checklist was used to evaluate the quality assessment of studies. Random effect model was applied to pool the prevalence data. Stata 13 software was used to analyze the data.

Results: Total of 58 eligible studies that fulfilled the inclusion criteria of the study were selected for qualitative synthesis. Among exotoxins; the highest prevalence was related to exoT (0.83 (CI95%: 0.64–0.96)). Lowest prevalence rate was seen in exoU with estimated prevalence 0.32 (CI95%: 0.24–0.41). In Carbapenem resistance isolates exoA and exoT had the highest prevalence (1.00 (CI95%: 0.98–1.00)).

Conclusion: This first meta-analysis on PA isolates with toxin potency indicated high prevalence of exotoxin production in clinical isolates of PA which is an alarming point as a clinical aspect. It was found that the ExoT has the most prevalence rate among toxins. The results of simultaneous evaluation of exotoxins and antimicrobial resistance can develop treatment policies against PA infections in hospitals and hospitalized patients.

1. Introduction

Bacterial toxins are involved in host-pathogen dialog by manipulating the host cell function especially in the site of bacterial infections. Most of pathogen bacteria which are able to secret toxins, target different cell types and promote infections and related diseases. Bacterial toxins are categorized in 7 different classes due to their nature and functions (Henkel et al., 2010). Up to now total of 44 bacterial toxins are introduced among Gram positive and negative bacteria. The bacterial toxins are conventionally classified as exotoxins and endotoxins according to either entrapped in the cell membrane or secreted out (Lahiri, 2000).

Pseudomonas aeruginosa (PA) as an opportunistic pathogen is the main cause of widespread and overwhelming hospital acquired infection due to an arsenal virulence factors such as toxins. Hence these factors are fundamental cause of increase morbidity and length of hospital stay in clinical settings (Yousefi-Avarvand et al., 2015). Responsible secretory virulence factors for *P. aeruginosa* are introduced in two main groups of enzymes and toxins. Identified enzymes as the name of Exoenzymes are exoY, exoS, exoT and exoU. Transmission of mentioned exoenzymes into eukaryotic cells is usually conducted by type III secretion systems (Engel and Balachandran, 2009). Exotoxin is another virulence factor which involved in tissue damage more than other introduced factors and also able to invade blood stream.Exotoxin A (encoded by the *tox*A gene) is the most introduced exotoxin in PA which is secreted by type II secretion system (Michalska and Wolf, 2015). According to literature reviews, it was found that 56.7% of *P. aeruginosa* strains in bacteremia cases are able to produce exotoxins. More over recent evidences demonstrated that *P. aeruginosa* strains which isolated from acute infections express more virulence factors than chronic infections like those afflicted individuals with cystic fibrosis. (Hauser, 2009) Distributions of PA toxin genes were found to vary as follow:

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Table 1
Characteristisc of studies included in the meta-analysis.

Author	Year	Country	*P.aeruginosa* Isolate	Exo A	Exo S	Exo U	Exo T	Exo Y
(Feltman et al., 2001)	2001	USA	55	–	35	21	55	46
(Berthelot et al., 2003)	2003	France	104	–	65	29	–	–
(Lanotte et al., 2004)	2004	France	81	–	50	–	–	–
(Garey et al., 2008)	2008	USA	119	–	86	31	–	–
(Badr et al., 2008)	2008	Egypt	47	42	–	–	–	–
(Wolska and Szweda, 2009)	2009	Poland	62	56	47	–	–	–
(Bradbury et al., 2010)	2010	Australia	24	–	21	5	24	24
(Mitov et al., 2010)	2010	Bulgaria	160	–	104	48	–	
(Strateva et al., 2010)	2010	Bulgaria	176	–	110	57	176	152
(Maatallah et al., 2011)	2011	Mediterranean countries	141	–	86	49	–	–
(Finlayson and Brown, 2011)	2011	India	17	–	11	–	–	5
(Idris et al., 2012)	2012	Malaysia	44	–	41	18	–	
(Jabalameli et al., 2012)	2012	Iran	96	–	28	62	96	91
(Agnello and Wong-Beringer, 2012)	2012	USA	270	–	152	103	–	
(Koutsogiannou et al., 2013)	2012	Greece	240	–	111	201	192	206
(Nikbin et al., 2012)	2012	Iran	286	243	154	–	–	–
(Doosti et al., 2012)	2012	Iran	70	66	–	–	–	–
(Holban et al., 2013)	2013	Romania	52	20	14	8	39	–
(Cotar et al., 2013)	2013	Romania	49	49	–	–	–	–
(Fazeli and Momtaz, 2014)	2014	Iran	102	36	69	14	37	23
(Peña et al., 2014)	2014	Spain	590	–	443	126	–	–
(Sabharwal et al., 2014)	2014	India	12	12	–	–	–	–
(Taee et al., 2014)	2014	Iran	70	57	–	–	–	–
(Ghadaksaz et al., 2015)	2014	Iran	104	88	–	–	–	–
(Corehtash et al., 2015)	2014	Iran	144	108	–	–	–	–
(Ferreira et al., 2015)	2015	Brazil	32	–	32	3	28	26
(Pobiega et al., 2016)	2015	Poland	26	–	23	5	23	25
(Pereira et al., 2015)	2015	Portugal	76	–	–	7	68	–
(Heidary et al., 2016)	2015	Iran	71	–	14	15	–	–
(Yousefi-Avarvand et al., 2015)	2015	Iran	156	141	104	102	–	–
(Joodzadeh et al., 2016)	2016	Iran	53	–	40	1	–	–
(de Almeida Silva et al., 2017)	2016	Brazil	35	–	25	5	–	–
(Hassuna, 2016)	2016	Egypt	66	–	42	–	–	–
(Fadhil et al., 2016)	2016	Iraq	286	28	27	–	–	–
(Najafi et al., 2015)	2016	Iran	160	–	42	83	8	88
(Firouzi-Dalvand et al., 2016)	2016	Iran	134	–	83	98	–	–
(Faraji et al., 2016)	2016	Iran	57	21	12	–	–	–
(Amirmozafari et al., 2016)	2016	Iran	102	81	61	–	–	–
(Khosravi et al., 2016)	2016	Iran	185	133	127	–	–	–
(Aditi et al., 2017)	2017	India	46	40	35	8	36	42
(de Oliveira and Pires, 2017)	2017	Brazil	48	–	26	13	–	–
(Sanchez-Diener et al., 2017)	2017	Spain	140	–	103	29	139	130
(Ahmadi et al., 2017)	2017	Iran	91	–	62	73	–	–
(Malek Mohamad et al., 2019)	2017	Iran	175	–	136	75	–	–
(Ahmed Jamal Hussein, 2017)	2017	Iraq	15	15	–	–	15	–
(Hanoon et al., 2017)	2017	Iraq	40	–	–	–	24	–
(Nahar et al., 2017)	2017	Bangladesh	18	10	9	–	–	–
(Ullah et al., 2017)	2017	Pakistan	54	18	20	–	–	–
(Bogiel et al., 2017)	2017	Poland	148	–	72	–	–	–
(Badamchi et al., 2017)	2017	Iran	84	58	–	–	–	–
(MORTAZAVI et al., 2017)	2017	Iran	91	79	–	–	–	–
(Zarei et al., 2018)	2018	Iran	58	23	13	11	–	–
(Zina Hashem Shehab, 2018)	2018	Iraq	55	–	51	24	–	–
(Pobiega et al., 2018)	2018	Poland	232	–	205	62	219	206
(Haghi et al., 2018)	2018	Iran	93	91	43	–	19	87
(Ahmad et al., 2018)	2018	Pakistan	182	156	175	–	–	–
(Jahromi et al., 2018)	2018	Iran	80	80	–	–	–	–
(Kainuma et al., 2018)	2018	Japan	41	5	–	36	–	–
Ellapen	2018	India	156		119			
Lee	2013	Korea	57	57	29	28	57	57
Takata	2018	Japan	49		15	34	49	46
Cho	2014	Korea	66		20	44		
Haghi	2018	Iran	31	31				

– It was not reported in articles.

exoS in 58–72%, *exoU* in 24%–42%, *exoY* in 89%, *exoT* in 92%–100% and toxA in 96% of isolates. Due to different study results around the world, exoT and exoY genes are the most express genes in PA isolates while exoS and exoU genes are found in limited number of isolates (Engel and Balachandran, 2009; Schulert et al., 2003). Apart from bacterial toxins, another problem related to *P. aeruginosa* and intensify the treatment of infection is antibiotics resistance. Although Carbapenems, especially meropenem and imipenem are effective drugs against gram negative bacteria, but an alarming increase in resistance rate against this choice group of antibiotics, have been reported from different regions of the world (Cho et al., 2014). Despite the presence of toxins in different clinical sources which have been investigated previously and reported differently, in this study, a systematic review and meta-analysis was conducted to estimate the overall prevalence of

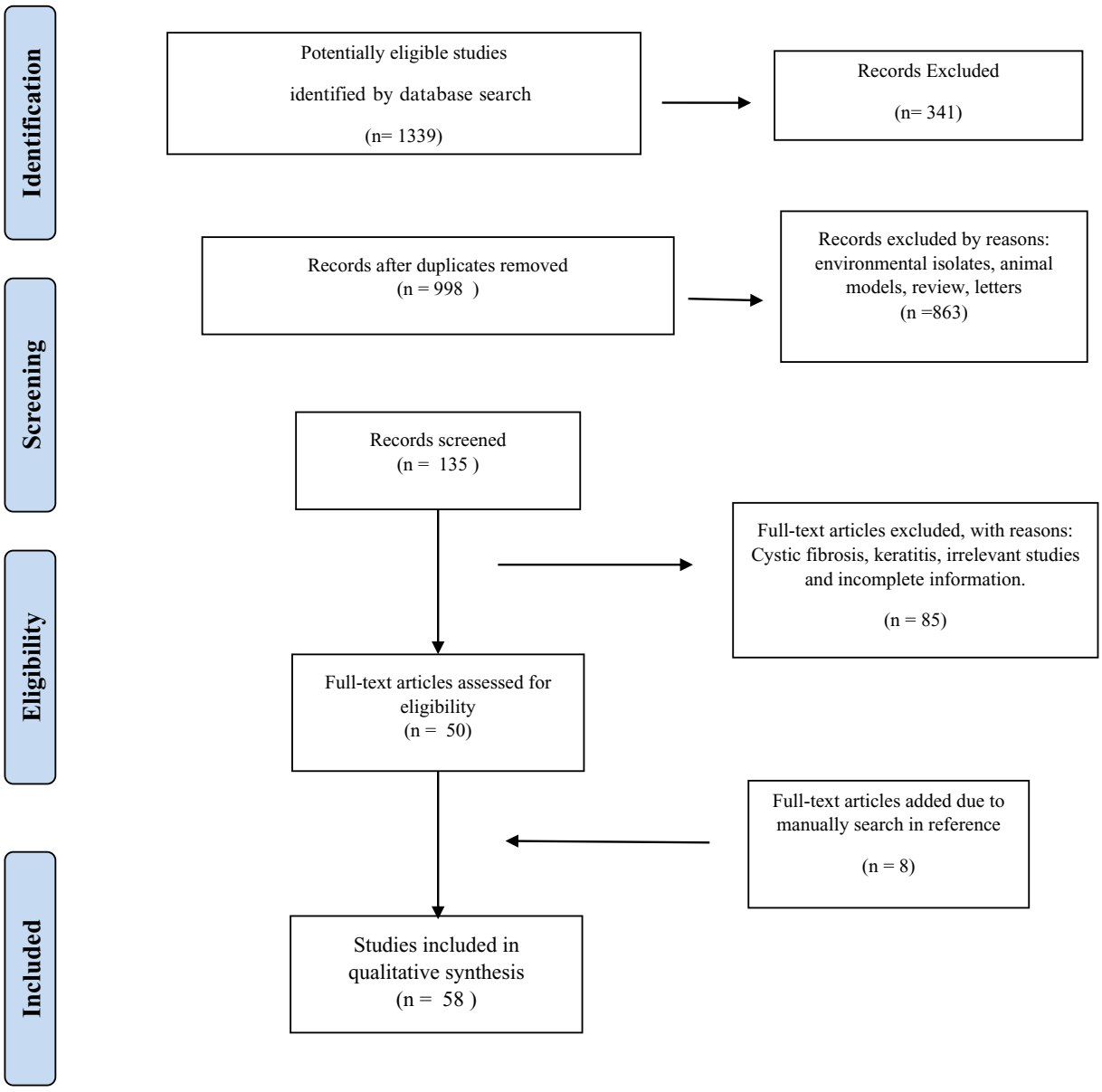

Fig. 1. PRISMA flow chart of systematic literature review and article identification.

toxins, and get insight about the pathogenicity of *P. aeruginosa* related to various distributions of toxins in hospitalized patients and also present the prevalence of toxins in Carbapenem resistant isolates.

2. Material and methods

2.1. Search strategy

The related protocol about this study was registered in PROSPRO; an international prospective register of systematic reviews (Registration code: CRD42019117273).

A comprehensive and systematic search was performed based on PRISMA guidelines checklist (preferred reporting items for systematic review and meta-analysis)(Moher et al., 2009). Related articles were identified through searching in the following database: MEDLINE, PubMed, EMBASE, Biosis Preview, ISI Web of Science, PsycInfo, CINHL and Scopus. Hence; the search terms choose based on Medical Subject Heading (Mesh) and consist "Exotoxins AND *Pseudomonas aeruginosa*" OR "ExoS AND *Pseudomonas aeruginosa*" OR "ExoU AND *Pseudomonas aeruginosa*" OR "ExoT AND *Pseudomonas aeruginosa*" OR "ExoA AND *Pseudomonas aeruginosa*" OR "ExoY AND *Pseudomonas aeruginosa*" OR "Virulence gene". Moreover, in order to find more eligible evidences and avoid to miss additional information a complementary search was fulfilled in reference lists of original articles to evaluate extra citations. The systematic search was limited to years 2000–2018.

2.2. Inclusion and exclusion criteria

Eligible articles for including in the meta analysis must have the following characteristics: a standard method for detecting clinical *Pseudomonas aeruginosa* in hospitalized patients, report data about the number of *P. aeruginosa* isolates, report the frequency of Exotoxins A, S, T, U and Y, also articles in English language. Studies in which isolates were from environmental, cystic fibrosis, keratitis, animal studies or reports other exotoxins were excluded. Furthermore; abstracts, conference papers, reviews, letters and short communications were not eligible for analysis.

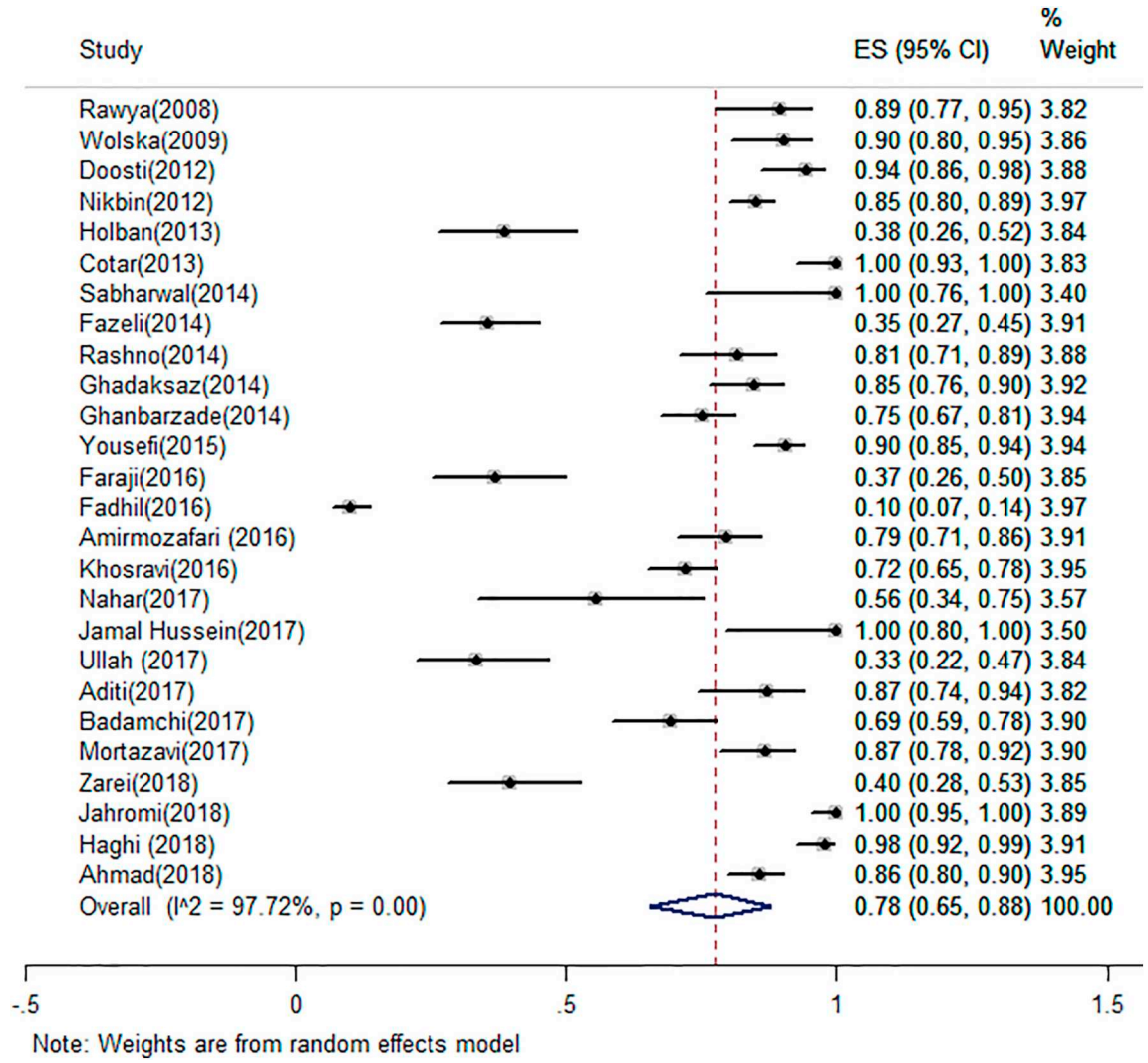

Fig. 2. Forest plot for prevalence of exoA in clinical isolates of *P. aeruginosa.*

2.3. Data extractions

Two reviewers extracted the data according to inclusion criteria and considering the following items: first author, publication year, study setting, total frequency of *P. aeruginosa* isolates, different exotoxins and the Carbapenem resistance isolates if possible. Table 1 provides the characteristics of recruited articles.

2.4. Quality assessment

For further emphasize the quality assessment of studies were conducted by two reviewers independently. Joanna Briggs Institute checklist was used for evaluation (Institute JB, 2017). Any disagreement in the findings unraveled with a third author discussion.

2.5. Statistical analysis

Inverse variance method was used to estimate the overall prevalence and related 95% CI of exotoxins in clinical isolates of *P. aeruginosa*. Statistical heterogeneity of included studies were explored by use of Chi-square test and I^2 statistics. The I^2 value > 50% or *p*-value < .05 were considered significant heterogeneity among studies and based on the results; either random or fixed effect method was used to estimate the pooled prevalence. Egger regression test and funnel plot was examined the publication bias. Stata 13 was applied for all analysis.

3. Results

3.1. Search results

Fig. 1 is shown the trial profile. Among 1339 studies in initial search; 341 records excluded due to duplication. 863 were subsequently excluded according to title and abstracts evaluation. We began the study selection by screening the full text of 135 remaining articles. Finally, 58 eligible studies included in qualitative synthesis which are provided in Table 1.

3.2. Prevalence of exotoxin

Twenty-six articles were reported exoA and based on the results, the overall pooled prevalence of exoA in *P. aeruginosa* isolates estimated 0.78 (0.65–0.88) with high and non-significant heterogeneity among studies (I^2 = 97.72%, P < .05). No publication bias was found visually in funnel plot (Fig. S1) and Egger's test (p = .32). Fig. 2 is provided for forest plot of exoA.

Most of the studies reported the prevalence of exoS (n- = 45) in *P. aeruginosa* isolates. The overall prevalence of exoS was 0.61 (0.54–0.68) (Fig. 3). Non-significant heterogeneity was seen among included articles (I^2 = 96.55%) with no publication bias (p = .84, Fig. S2 in supplementary).

In order to estimate the overall prevalence of exoT, seventeen

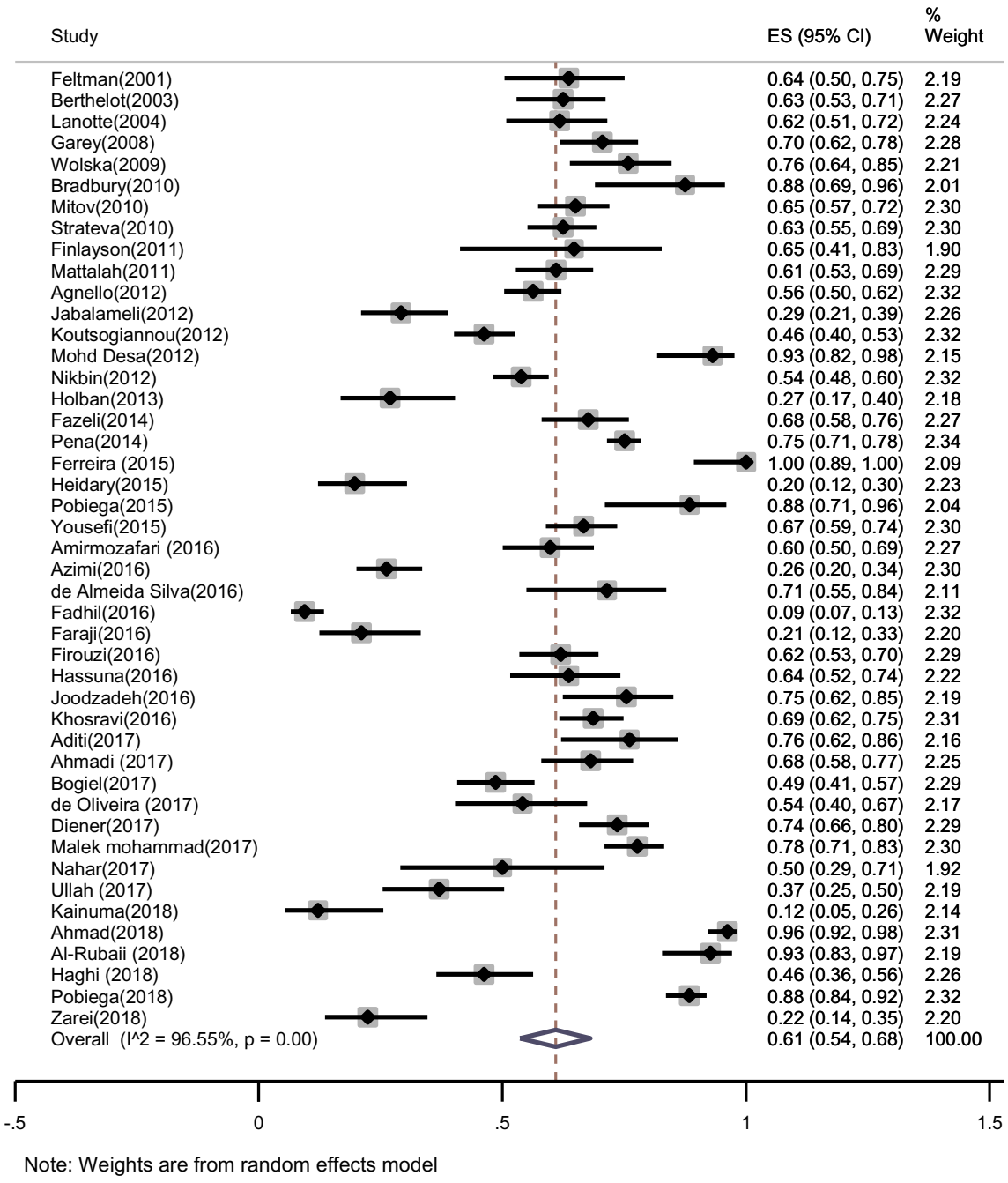

Fig. 3. Forest plot for prevalence of exoS in clinical isolates of *P.aeruginosa.*

articles were appraised and the pooled rate was reported 0.83 (0.64–0.96). Among other exotoxins the highest prevalence was related to exoT. Fig. 4 is shown the forest plot of this toxin. Due to high heterogeneity among studies (I^2 = 98.58%), random effects model was used, also funnel plot visually and Eggers regression test did not show any publication bias (P = .86). Sensitivity analysis were performed by excluding three articles (Fazeli and Momtaz, 2014; Najafi et al., 2015; Haghi et al., 2018) but no notable decrease was seen in attributed heterogeneity (I^2 = 92.70%).

Fourteen articles were evaluated to estimate the overall prevalence of exoY and it is reported 0.82 (0.71–0.91) which has been showed in Fig. 5. There was high and non-significant heterogeneity among included studies (I^2 = 95.88, P < .05). A sensitivity analysis was conducted by excluding 3 articles (Finlayson and Brown, 2011; Fazeli and Momtaz, 2014; Najafi et al., 2015), and a conspicuous decrease was seen in attributed heterogeneity (P = .02, I^2 = 54.11%) but the prevalence rate of exoY increase to 0.91 (0.88–0.93).

Reported results about prevalence of exoU was found in 34 studies,

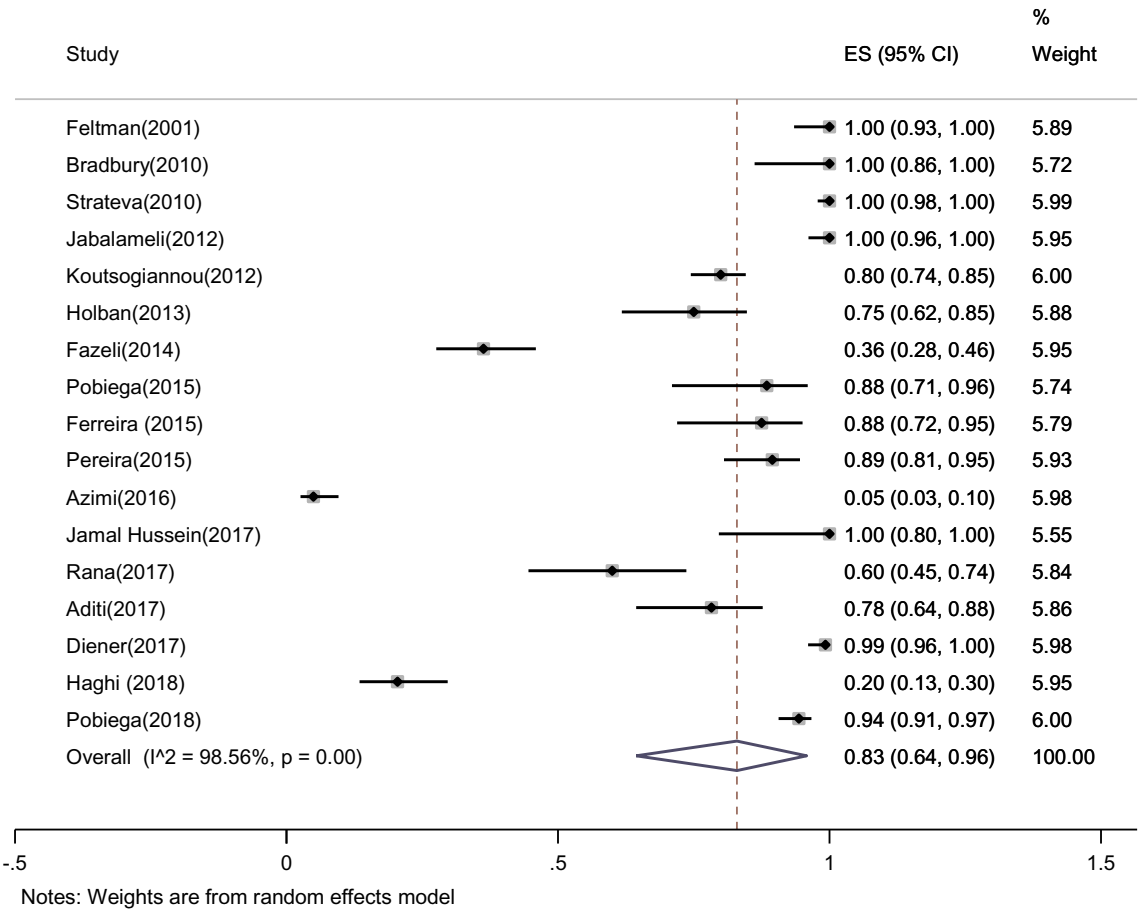

Fig. 4. Forest plot for prevalence of exoT in clinical isolates of *P.aeruginosa.*

and the pooled estimation via random effects model was 0.32 (0.24–0.41) which is the lowest prevalence rate among other toxins (Fig. 6). High and non-significant heterogeneity was seen among studies. Visually results about publication bias are presented in Fig. 3S.

Overall a summary of exotoxin prevalence, heterogeneity and publication bias test are provided in Table 2.

3.3. Exotoxin in Carbapenem resistance Pseudomonas aeruginosa

To fill the knowledge gaps about the prevalence of exotoxin genotype among Carbapenem resistance *P.aeruginosa* isolates, we did a specific random effect analysis in non-susceptible toxin strains (Table 2). It was only possible to conduct a pooled analysis for Carbapenem resistance for five available studies which are presented in Table 3. Some articles excluded due to report the resistance rate among exotoxins (Garey et al., 2008).

4. Discussion

Virulence factors are important features that increase the threaten role of infectious agents especially in hospitalized patients. Virulence factors besides resistant capability can increase the risk of mortality and morbidity in hospital settings. By considering these items knowing the ability of nosocomial infections will be helpful in infectious control programs.

In nosocomial infections, *Pseudomonas aeruginosa* is one of the important and opportunistic pathogen which is going on to be worldwide concern. The ability of this bacterium in virulence factors producing, make it more danger for hospitalized patients. Beside different virulence factor groups of this bacterium, Endo and Exo toxins are more remarkable. Since various results shown different prevalence of toxins in *P. aeruginosa* isolates and there was no comprehensive analysis about this controversy, current meta-analysis study was conducted to estimate the incidence of toxins in hospital isolates of *P. aeruginosa* which has worldwide outbreak. According to the pooled estimation of included articles, it has been conducted that ExoT is the most prevalent virulence factor (83%) in *P. aeruginosa* isolates. ExoY and ExoA are two other toxins which are the most prevalent studied virulence factors have been presented in the worldwide isolates. In many studies, ExoT introduced as a toxin factor which is produced in the most clinical isolates. Exoenzyme T is a protein with bifunctional activity. This enzyme has an amino-terminal G-protein-activating protein (GAP) domain which can target small Rho-like GTPases and cause cytoskeletal rearrangements. (Barbieri, 2000; Garrity-Ryan et al., 2000; Goehring et al., 1999; Krall et al., 2000) ExoT can target two host kinases; CrKI ans CrkII, which regulate focal adhesion and phagocytosis (Sun and Barbieri, 2003).

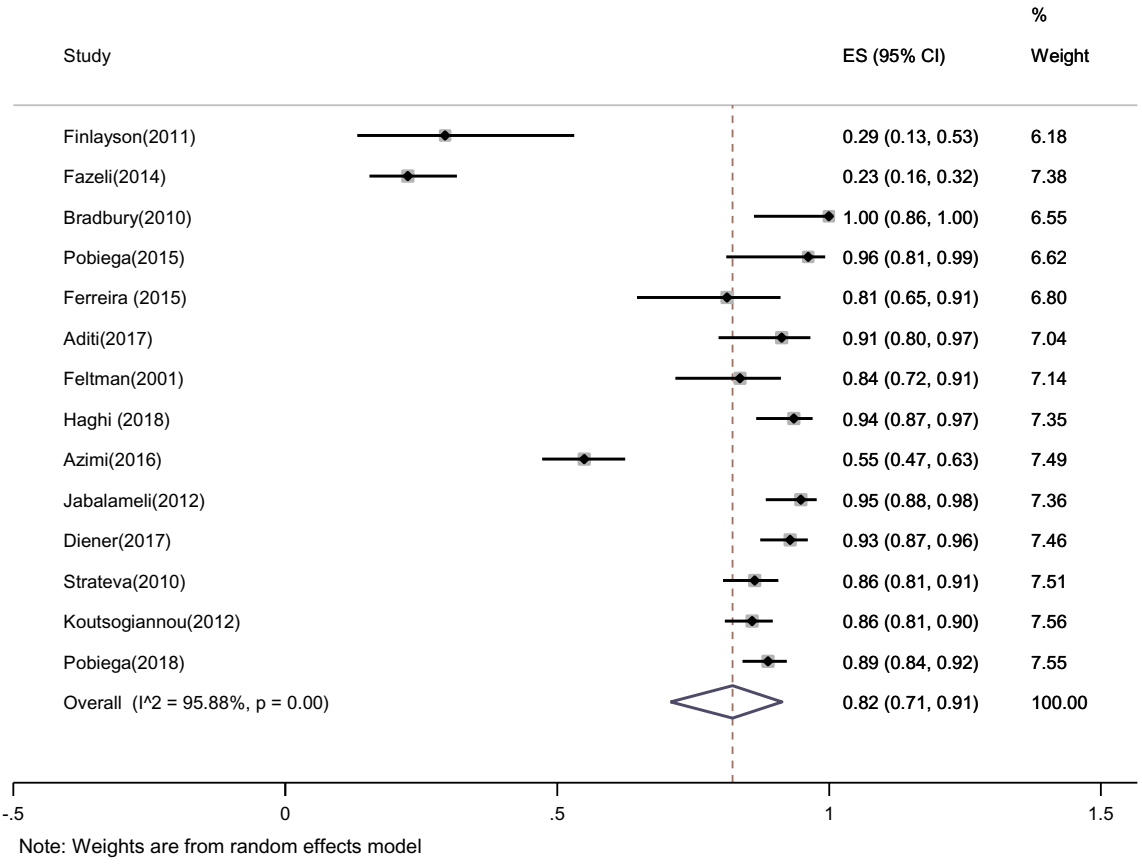

Fig. 5. Forest plot for Prevalence of exoY in clinical isolates of *P.aeruginosa.*

Another prevalent toxin in clinical *P. aeruginosa* isolates is ExoY, which is an adenylate cyclase. This toxin can raise the intracellular cAMP levels in mammalian cells and causes actin cytoskeleton restructuring (Barbieri and Sun, 2004). Another important *P. aeruginosa* toxin is ExoA which is responsible for tissue necrosis. This toxin inhibits protein biosynthesis by transferring an ADP-ribosyl moiety to elongation factor II of mammalian cells. Based on the functionality of these toxins which mentioned above, these potentially will increase the pathogenicity risk of this bacterium. Although the results of this meta-analysis on worldwide clinical isolates of *P. aeruginosa* showed that ExoT, ExoY and ExoA are the most prevalent respectively, but in some clinical studies different records have been reported for these toxins (i.e. 90–95% for ExoA)(Ban et al., 2014; Matar et al., 2002). This controversy results may be due to the diversity in the type of samples, strain types and geographical area of surveys (Malek Mohamad et al., 2019). According to the various specifications of bacteria and different distributions of virulence genes in the bacterial populations, either this can cause some *P. aeruginosa* strains adapted better to the specific conditions found in specific infectious sites and patients (Sabharwal et al., 2014). Despite existence of toxins in *P. aeruginosa* isolates; another problem that make remedial options more hard; is the resistance of these isolates to routine antimicrobial agents. Whereas Imipenem and Meropenem are the main therapeutic choice drugs for *P. aeruginosa* infection; high incidences of Carbapenem resistance isolates become general concern in worldwide. As it was shown in current results; most of resistance genotypes harbor studied toxins. Between mentioned toxins, ExoA and ExoT were the most prevalent virulence factors in resistant clinical isolates of *P. aeruginosa*. According to the selection pressure of antimicrobial agents by over prescription of antibiotics in hospital settings, and accompaniment of resistant criteria with Exotoxins simultaneously, these make the management therapy and infectious control with more challenges.

As far as we know, this is the first meta-analysis investigation on toxin prevalence over all the clinical isolates of *P. aeruginosa*; the results indicated no publication bias. The inclusion of studies from different countries and acute infections from different sources (blood, urine, wound, burn wound, etc) allowed the results to be generalized between clinical isolates. Nevertheless some limitations could not be ignored. High statistical heterogeneity was found between studies, which might be due to patient's demographic, history and study settings. Either most of studies did not report the prevalence of toxins distinctly in clinical isolates and it was difficult to distinguish the exact information of the study. Either as a suggestion, evaluation of other virulence factors can be useful as a prognostic factor for control of sever *P. aeruginosa* infections in hospital settings.

5. Conclusion

P. aeruginosa has become one of the leading causes of hospital-acquired infections. Due to recent results, variety of virulence factors especially toxins are present in *P. aeruginosa* isolates and high prevalence of them is an alarming point for clinicians. As a conclusion assessment of antimicrobial resistance, this will help clinicians in directing clinical management of patients and develop treatment policies against *P. aeruginosa* infections.

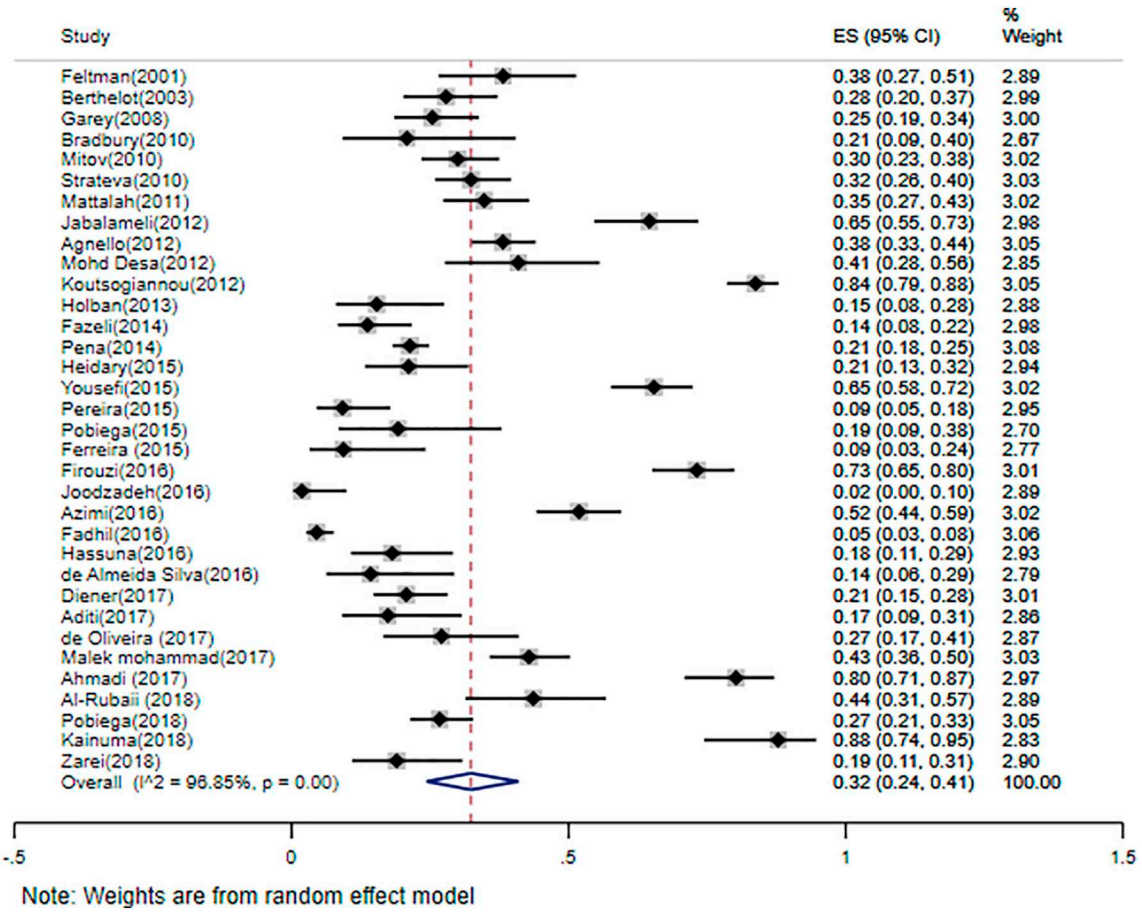

Fig. 6. Forest plot for prevalence of exoU in clinical isolates of *P.aeruginosa.*

Table 2
Summary of exotoxin prevalence.

Exotoxin	No. of studies	Prevalence (95%CI)	I^2%	Heterogeneity test		Egger test	
				Z	P	t	P
ExoA	26	0.78 (0.65–0.88)	97.72	14.91	P < .05	1.00	0.326
ExoS	45	0.61 (0.54–0.68)	96.56	22.23	P < .05	−0.19	0.84
ExoT	17	0.83 (0.64–0.96)	98.58	10.05	P < .05	0.18	0.86
ExoU	34	0.32 (0.24–0.41)	96.85	12.30	P < .05	−0.03	0.98
ExoY	14	0.82 (0.71–0.91)	95.88	15.78	P < .05	−0.25	0.80

Table 3
Prevalence of exotoxin in Carbapenem resistance *Pseudomonas aeruginosa.*

Exotoxin	No. of studies	Prevalence of Exotoxin (95%CI)	I^2%	Heterogeneity test		Egger test	
				Z	P	t	P
Exo A	2	1.00 (0.98–1.00)	–	26.77	P < .05	–	–
Exo S	4	0.48 (0.23–0.72)	95.02	5.40	P < .05	−3.18	0.086
Exo T	2	1.00 (0.98–1.00)	–	29.67	P < .05	–	–
Exo U	3	0.62 (0.49–0.740	63.67	13.23	P < .05	0.07	0.95
Exo Y	2	0.98 (0.94–1.00)	–	27.79	P < .05	–	–

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Ethical approval

All of the study processes were approved by the ethics committee code 97-01-106-18801 from Shiraz University of Medical Sciences.

Informed consent

Not applicable.

Author contributions

Amir Emami, Marokh Rajaee, Neda Pirbonyeh design the study, collecting the data and monitoring the data collection. Fatemeh Javanmardi statistical analysis plan, cleaned and analyzed the data and implemented the study. Amir Emami and Abdolkhalegh Keshavarzi wrote the manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.104037.

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