



Antimicrobial prescriptions in cancer patients: An evaluation based on clinical and pharmacotherapeutic indicators

[Prescripción de antimicrobianos en pacientes oncológicos: una evaluación basada en indicadores clínicos y farmacoterapéuticos]

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Abstract

Context: The inappropriate use of antimicrobials is a public worldwide health problem that causes increased morbidity and mortality, bacterial resistance, and care costs and hospitalizations.

Aims: To evaluate the prescription of antimicrobials in patients treated at a Cuban cancer hospital, based on clinical indicators and pharmacotherapeutics.

Methods: Retrospective descriptive study of prescription-indication of antimicrobials, during the period of January-December 2018, in a Cuban cancer hospital. Hospitalized patients with antimicrobial treatment were included in the study. The determination of prevalent microorganisms and bacterial resistance was analyzed through the book of infection control and antibiograms. The adequate and inadequate categories of prescriptions based on clinical and pharmacotherapeutic indicators were established to evaluate the prescription.

Results: In a sample of 50 patients, laryngeal cancer (40%), surgical wound infection (48%), and beta-lactam pharmacological group (46.4%) prevailed. Eight germs were isolated, with a predominance of *Pseudomonas aeruginosa* (26.3%) with gentamicin resistance (66.7%). *Staphylococcus* spp. presented resistance to ciprofloxacin (63.6%) and amikacin (54.5%). *Escherichia coli* exhibited resistance to gentamicin (66.7%) and ciprofloxacin (55.6%). Appropriate prescriptions predominated (52%), with the indicator 'indication and therapeutic scheme' being the most difficult.

Conclusions: The inappropriate use of antimicrobials constitutes a problem in cancer patients and highlights the need to implement actions that contribute to raising the quality of prescriptions to prevent microbial resistance.

Keywords: antimicrobials; bacterial resistance; clinical indicators; drug use studies; oncological diseases.

Resumen

Contexto: El uso inadecuado de antimicrobianos es un problema de salud pública a nivel mundial que provoca aumento de la morbimortalidad, resistencia bacteriana y costos de atención y hospitalizaciones.

Objetivos: Evaluar la prescripción de antimicrobianos en pacientes atendidos en un hospital oncológico cubano, con base en indicadores clínicos y farmacoterapéuticos.

Métodos: Estudio descriptivo retrospectivo de prescripción - indicación de antimicrobianos, durante el período enero - diciembre de 2018, en un hospital oncológico cubano. Se incluyeron en el estudio pacientes hospitalizados con tratamiento antimicrobiano. La determinación de microorganismos prevalentes y resistencia bacteriana se analizó a través del libro de control de infecciones y antibiogramas. Se establecieron las categorías de prescripción adecuada e inadecuada en base a indicadores clínicos y farmacoterapéuticos para evaluar la prescripción.

Resultados: En una muestra de 50 pacientes predominó el cáncer de laringe (40%), la infección de herida quirúrgica (48%) y el grupo farmacológico betalactámico (46,4%). Se aislaron ocho gérmenes, con predominio de *Pseudomonas aeruginosa* (26,3%) con resistencia a gentamicina (66,7%). *Staphylococcus* spp. presentó resistencia a ciprofloxacina (63,6%) y amikacina (54,5%). *Escherichia coli* mostró resistencia a gentamicina (66,7%) y ciprofloxacina (55,6%). Predominó la prescripción adecuada (52%), siendo el indicador 'indicación y esquema terapéutico' lo más difícil.

Conclusiones: El uso inadecuado de antimicrobianos constituye un problema en pacientes oncológicos y destaca la necesidad de implementar acciones que contribuyan a elevar la calidad de las prescripciones para la prevención de resistencias microbianas.

Palabras Clave: antimicrobianos; enfermedades oncológicas; estudios de uso de drogas; indicadores clínicos; resistencia bacteriana.

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INTRODUCTION

Cancer is a devastating disease, and it is a major cause of death in many countries worldwide. In the last decades, there has been an increase in the incidence of this disease, constituting today the second cause of death behind cardiovascular diseases (Siegel et al., 2016; Suarez García et al., 2013). In Cuba, deaths from malignant tumors are also the second leading cause of death, after heart disease, with a rate of 223.0 per 100 000 inhabitants (MINSAP, 2020).

Patients with malignancies are at risk of infectious diseases, and complications due to infections are a serious cause of morbidity and mortality in this patient (Fuentes et al., 2014; Zembower, 2014). The inappropriate use of antimicrobials is a health worldwide problem because it causes an increase in morbidity and mortality and contributes to an increase in bacterial resistance and costs of care, causing unnecessary adverse effects, prolonged hospitalizations, and infections associated with health care (Aitken et al., 2019; Cernuda Mesa et al., 2017). The literature indicates that high resistance levels are found in bacteria isolated from settings with a high density of antibiotics. For example, intensive care units and the prolonged use of antimicrobials increase the risk of infection by resistant organisms (Cantón et al., 2013; Resurrección-Delgado et al., 2020).

A study developed by Mir Narbona et al. (2009) in two hospital services in Havana city, Cuba, identified deficiencies in the prescription of antimicrobials fundamentally attributed to the use of antimicrobials without evidence of infection, to the wrong choice of treatment and the wrong preventive indication. Similarly, Rodríguez et al. (2017) reported the inadequate antimicrobial prescriptions in a study conducted at a general hospital in the Contramaestre municipality in Santiago de Cuba, Cuba.

In Cuba, few studies document the use of antimicrobials in cancer patients. Most of the registered investigations determine the antimicrobial's frequency use (Rubio Hernández et al., 2001), the most frequent germs, and the clinical epidemiological characterization of the studied sample (Céspedes Quevedo et al., 2010). However, they did not evaluate the prescription or address antimicrobial resistance in these types of patients.

Therefore, based on clinical and pharmacotherapeutic indicators, the present work was developed to evaluate the prescription of antimicrobials in patients treated at a Cuban cancer hospital.

MATERIAL AND METHODS

A retrospective descriptive study was conducted from January to December 2018 in an Oncology Hospital with the methodology of a study on the use of prescription-indication medications with elements of therapeutic schemes. All hospitalized patients from all units of the healthcare center who received antimicrobial treatment and were reported in the official infection book were included. Those patients with incomplete medical records were excluded. The research was approved by the Scientific Council of the Hospital Center (PA-1803), guaranteeing the confidentiality of the data for each patient.

The sample was characterized through biosocial variables (sex, age), clinical variables (underlying disease, associated diseases, type of infection) and therapeutic variables (type of antimicrobial). The information necessary to carry out the research was obtained through reviewing the patients' medical records and the official infection book. To determine the prevalent microorganisms, and the behavior of bacterial resistance, information was considered when evaluating the indication of antimicrobials. A review of the hospital's microbiology laboratory records was carried out, including the official infection book of the Oncology Hospital and the results of the antibiograms performed on the strains isolated from the samples processed during 2018.

To evaluate the prescription of antimicrobials, the following indicators were analyzed:

Indication and therapeutic scheme: According to what is confirmed in the literature of the established clinical guidelines and updated protocols, the antimicrobial indicated empirically for surgical prophylaxis. For the therapeutic scheme, the dose, frequency, route of administration and duration of treatment were taken into account (Gilbert et al., 2013; MINSAP, 2014; Vilar-Compte et al., 2011). In addition, treating the infectious process was considered for the evaluation of the indication and the behavior followed by the doctor once the results of the microbiological culture and the antibiogram were received.

Individualization of treatment: When prescribing, the physician considered comorbidity, drug dose, and antimicrobial contraindications, as well as the results of clinical laboratory tests.

Antimicrobial combinations: The prescription of antimicrobial drug combinations was taken into account since it would allow greater effectiveness than using them separately (due to additive effect or synergism) or used to treat serious polymicrobial infections and/or of unknown etiology.

It was considered:

- Adequate prescription: When the indication and the therapeutic scheme, the individualization of the treatment and the antimicrobial combinations were adequate, according to the criteria established above.

- Inadequate prescription: When at least one of the aforementioned aspects was breached.

The data processing was conducted through a descriptive statistical analysis using as a summary measure the calculation and comparison of percentages using Microsoft Excel 2019 (v19.0).

RESULTS

The universe object under study consisted of 57 patients and the sample was made up of 50 patients who met the established requirements. Seven patients were excluded because the medical records were incomplete. The variables that allowed the characterization of the sample are presented in Table 1. Male patients (58%) and those older than 60 years (54%) predominated. Regarding the underlying disease, laryngeal cancer prevailed (40%), followed by breast cancer (30%). A predominance of diabetes mellitus (73.7%) was detected among the patients' associated diseases, followed by arterial hypertension (50%). The type of infection that prevailed was the surgical wound (48%), followed by respiratory infections (30%). Concerning the therapeutic variable type of antimicrobial, the most used pharmacological groups were beta-lactams (46.4%) and aminoglycosides (17.8%).

From the determinations conducted, a total of eight germs were isolated, and Gram-Negative bacteria predominated (73.7%), of which the most prevalent was *Pseudomonas aeruginosa* (26.3%), followed by *Staphylococcus* spp. (19.2%) and *Escherichia coli* (15.7%), as shown in Table 2.

Table 3 exhibits the antimicrobial susceptibility behavior of the most prevalent germs. *Pseudomonas aeruginosa* showed 66.7% resistance to gentamicin and 46.7% to ciprofloxacin. *Staphylococcus* spp. showed resistance against ciprofloxacin (63.6%) and amikacin (54.5%), and *Escherichia coli* showed 66.7% resistance to gentamicin and 55.6% to ciprofloxacin.

Appropriate prescriptions (75.6%) predominated over inappropriate ones (24.4%). Among the indicators used to evaluate antimicrobials' prescription, the highest percentage of adequate prescription (80.0%) was the individualization of treatment indicator. Moreover, the indicator 'indication and therapeutic scheme' was the ones that presented the greatest difficulties, exhibiting 24% of inappropriate prescriptions. These results are reflected in Table 4.

DISCUSSION

Malignant neoplasms are 1.5 times higher in men than in women and are considered typical of the elderly since more than 65% of all these occur in this group, representing 20.8% of the Cuban population. The risk of cancer increases with age because there is a longer exposure period to many agents. In aging, there are physical and mental changes that cause diseases, which in relation to other factors, such as smoking and alcoholism, make the elderly more susceptible to neoplasms (MINSAP, 2020; Siegel et al., 2016). The above analysis explains the predominance of the male patients and those over 60 years of age in the research conducted. These results were similar to those reported by Pérez Delgado et al. (2014) and are in agreement with the reports of the Statistical Yearbook of Health in Cuba, which refers to a higher incidence of cancer in male patients (MINSAP, 2020).

In the study mentioned above, the prevalence of laryngeal neoplasms followed by breast neoplasms coincided with what was published by Céspedes Quevedo et al. (2010). It is related to the Statistical Yearbook of Health reports, which describes a high incidence of these neoplasms in the Cuban population, with a predominance of laryngeal cancer in men and breast cancer in women (MINSAP, 2020). Hypertension and diabetes mellitus are highly prevalent in Cuba, which is higher in older adults than in other age groups (MINSAP, 2020). This explains the predominance of these diseases in the sample, which are similar to those reported by Pereira Relis et al. (2016).

Surgical wound infection directly impacts the patient's prognosis. It has serious consequences, including an increase in expenses due to their treatment and an increase in hospitalization time (Carvalho et al., 2017). Céspedes Quevedo et al. (2010) reported the prevalence of this type of infection and could be related to risk factors such as body mass index, smoking, associated diseases, the duration of surgery and the length of hospitalization. There are long-term oncological surgeries in which the patient has a greater wound exposure to pathogens, and the hospitalization time constitutes another important factor of incidence due to the greater probability of colonization in the patient's wound during the period of hospitalization, which facilitates the installation of infectious processes (Pérez-Martínez et al., 2014). Another incidence factor is respiratory infections, which are a frequent reason for consultation and hospitalization in cancer patients and are associated with high morbidity and mortality (Fuentes et al., 2014). This explains its predominance in the patients in the sample.

Table 1. Characterization of the sample.

Variable			Patients	
			N	%
Biosocials	Sex	Female	21	42
		Male	29	58
	Age	30-39	9	18
		40-49	7	14
		50-59	7	14
Clinics	Underlying diseases	60 and over	27	54
		Laryngeal cancer	20	40
		Breast cancer	15	30
		Prostate cancer	5	10
		Thyroid cancer	4	8
		Nasopharyngeal cancer	3	6
	Associated diseases	Other	3	6
		Arterial hypertension	25	50
		Mellitus diabetes	15	30
		Bronchial asthma	6	12
	Infection types	Renal insufficiency	4	8
		Surgical wound infection	24	48
		Respiratory infection	15	30
		Urinary infection	6	12
Therapeutics	Antimicrobial types	Other	5	10
		Beta-lactams	39	46.4
		Aminoglycosides	15	17.8
		Quinolones	13	15.5
		Metronidazole	9	10.7
		Tetracyclines	3	3.6
	Others	5	6.0	

Source: Clinical history.

Table 2. Prevalence of isolated microorganisms.

Microorganism	Classification	No.	%
<i>Pseudomonas aeruginosa</i>	Gram Negative	15	26.3
<i>Staphylococcus</i> spp.	Gram Positive	11	19.3
<i>Escherichia coli</i>	Gram Negative	9	15.8
<i>Proteus</i> spp.	Gram Negative	8	14.0
<i>Enterobacter</i> spp.	Gram Negative	4	7.0
<i>Enterococcus</i> spp.	Gram Negative	4	7.0
<i>Acinetobacter</i> spp.	Gram Negative	3	5.3
<i>Klebsiella</i> spp.	Gram Negative	3	5.3
Total		57	100

Source: Official infection report book

Table 3. Antimicrobial susceptibility of the most frequently isolated strains.

Antimicrobial	Microorganism								
	<i>Pseudomonas aeruginosa</i>			<i>Staphylococcus spp.</i>			<i>Escherichia coli</i>		
	S N (%)	R N (%)	TP N (%)	S N (%)	R N (%)	NT N (%)	S N (%)	R N (%)	NT N (%)
Amikacin	11 (73.3)	3 (20.0)	1 (6.7)	3 (27.3)	6 (54.5)	2 (18.2)	8 (88.8)	1 (11.1)	-
Gentamicin	3 (20.0)	10 (66.7)	2 (13.3)	7 (63.6)	3 (27.3)	1 (9.1)	1 (11.1)	6 (66.7)	2 (22.2)
Piperacillin	5 (33.3)	4 (26.7)	6 (40.0)	NT	NT	NT	NT	NT	NT
Ciprofloxacin	1 (6.7)	7 (46.7)	7 (46.7)	4 (36.4)	7 (63.6)	-	1 (11.1)	5 (55.6)	3 (33.3)
Doxycycline	NT	NT	NT	10 (91.0)	-	1 (9.0)	NT	NT	NT
Aztreonam	4 (26.7)	2 (13.3)	9 (60.0)	NT	NT	NT	NT	NT	NT
Fosfomicin	1 (6.7)	3 (20.0)	11 (73.3)	8 (72.7)	2 (18.1)	1 (9.0)	7 (77.8)	1 (11.1)	1 (11.1)
Amoxicillin/sulbactam	NT	NT	NT	8 (72.7)	-	3 (27.2)	NT	NT	NT
Azithromycin	NT	NT	NT	5 (45.4)	1 (9.0)	5 (45.4)	NT	NT	NT
Cefotaxime	NT	NT	NT	NT	NT	NT	3 (33.3)	1 (11.1)	5 (55.6)
Ceftriaxone	NT	NT	NT	NT	NT	NT	1 (11.1)	2 (22.2)	6 (66.7)

S: Sensitivity; R: Resistance; NT: Non-Tested. Source: Microbiology laboratory records (official infection report book).

Table 4. Indicators to evaluate the prescription of antimicrobials.

Indicators	Adequate		Inadequate		Total N
	N	%	N	%	
Indication and therapeutic scheme	57	76.0	18	24.0	75
Individualization of treatment	60	80.0	15	20.0	75
Drug combinations	10	13.3	8	10.7	18
Total	127	75.6	41	24.4	168

Source: Clinical history.

Antimicrobials are among the most widely used therapeutic groups and account for more than 60% of drug expenses in hospitals (García et al., 2013). In the present investigation, cephalosporins were the most used β -lactams, followed by aminoglycosides, which coincides with the findings reported by García et al. (2013). The excellent safety profile, the greater microbial coverage that can be achieved with third-generation cephalosporins in empirical treatments, and the use of cefazolin, a first-generation cephalosporin, in surgical prophylaxis as the antibiotic of choice (Espinosa et al., 2008; García et al., 2013), could explain the increased use of this group of drugs.

Other authors have also reported the prevalence of gram-negative bacteria isolation in the hospital environment. In particular, the prevalence of non-fermenting bacilli such as *Pseudomonas aeruginosa* and gram-positive germs such as *Staphylococcus spp.* was also reported by Espinosa et al. (2008). However, when comparing these results with those obtained in

other hospitals (Hernández et al., 2016; Martínez et al., 2013), differences were found in the prevalence of the different bacteria, possibly attributed to the type of patients and their diseases, geographic location and climate, as well as the mechanisms of control and prevention of each one of them, as reported by Martínez et al. (2013). *Pseudomonas aeruginosa* is a frequently isolated pathogen; people with burns, with immunodeficiencies, and hospitalized patients and cancer patients are population groups susceptible to infections by this microorganism (Gudiol and Caratala, 2014).

At present, although the highest mortalities remain associated with infections by Enterobacteriaceae (*E. coli*, *Proteus spp.*, *Klebsiella spp.*) and *Pseudomonas aeruginosa*, the frequency of gram-positive infections clearly exceeds the previous ones (Gudiol and Caratala, 2014). Gram-positive bacteria include species of the genus *Staphylococcus*. Among those of greatest clinical importance is *Staphylococcus aureus*, which has

become the main cause of infections in the circulatory stream, being among the most frequent complications in immunosuppressed patients with cancer and associated with high morbidity, mortality and costs. *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* are commonly responsible for device-related infections and urinary tract infections, these being less infectious than *S. aureus* (Gudiol and Carratala, 2014).

One of the most important problems affecting public health in most countries is the growing resistance to antimicrobials by their misuse and other factors that have led to resistant strains. Studying the antimicrobial susceptibility profiles of microorganisms that cause infections is very important in the hospital environment as it allows obtaining updated information to identify potential therapeutic options and design antibiotic control and rationalization programs (Hernández et al., 2016).

The considerable resistance of *Pseudomonas aeruginosa* to gentamicin found in the research reveals the need to monitor its behavior, considering that it is a microorganism with a great capacity to acquire antimicrobial resistance mechanisms, easily dispersed and persistent in nosocomial environments (Horcajada et al., 2019; Wu et al., 2021). These findings differ from those reported by other authors in Cuba (Cabrera et al., 2014; Monté and Martínez, 2017), which is explained by taking into account that bacterial isolates of *Pseudomonas aeruginosa* can present diverse resistance patterns and can reach high rates of resistance to multiple antimicrobials (Kim et al., 2017).

In the case of *Staphylococcus* spp. and *Escherichia coli*, the behavior obtained is possibly related to the greater availability and use of gentamicin, amikacin, and ciprofloxacin in hospitalized patients. Hence, these drugs must be used with care and with the support of microbiological results to avoid the increase and spread of bacterial resistance. In the present study, it is important to note that the highest antimicrobial resistance values found among the isolated microorganisms with the highest prevalence were to aminoglycosides (gentamicin and amikacin) and ciprofloxacin, the most widely used drugs according to the findings of this research. In the vast majority of the strains, it was impossible to determine the resistant behavior of the isolated germs against beta-lactams due to the lack of discs, which did not allow us to compare their behavior with that of other investigations.

The inappropriate use of antimicrobials is a complex problem involving multiple interrelated factors: cultural and social beliefs, knowledge and attitudes, infrastructure and economic interests (Seah et al., 2017). In the research conducted, lower values of in-

appropriate prescription were obtained than those reflected in the study by Rodríguez et al. (2017). In their research, 82.3% of the prescriptions of antimicrobials in the hospital environment were inappropriate. These differences may correlate to the knowledge and attitudes of the hospital prescribers, the basic table of medications established in each institution, and the antimicrobial policy regulated by the hospital institution for the type of patient. It must also be taken into account the fact that the present investigation was developed in a hospital center located in the provincial capital, which is a highly complex institution with specialized and experienced professionals dedicated to hospital care, research, teaching, and the provision of services to the provinces of the eastern region of the country.

The greatest difficulties found in the evaluated prescriptions were in the indications and therapeutic regimens, which coincides with what is reported in the literature (Seah et al., 2017). They were attributed to the incorrect indication and the untimely administration of the antimicrobial in surgical prophylaxis. In this regard, the literature (Vilar-Compte et al., 2011) indicates that the prescription of antimicrobials in surgical prophylaxis is suboptimal in most hospitals and reveals an inappropriate antimicrobial agent selection untimely infusion and unnecessary continuation of the antibiotic are the most frequent problems. It also suggests that the infusion of the antimicrobial 20-60 minutes before the incision is the most important factor for surgical prophylaxis to reduce the frequency of infection in the surgical wound. This could explain the prevalence of surgical wound infection in the patients in the sample.

The study identified the use of ceftazidime in surgical prophylaxis, which is not recommended since it is not more effective than first-generation cephalosporins against *Staphylococcus* and usually has coverage against bacteria that are unlikely in the operative field (*Pseudomonas*). In addition, they generate selective pressure on germs such as *Enterococcus* and can increase the appearance of resistant strains such as extended-spectrum beta-lactamase (Vilar-Compte et al., 2011).

The study also detected the use of broad-spectrum antimicrobials such as meropenem without justification, instead of using narrow-spectrum antimicrobials as suggested by the literature (Monté and Martínez, 2017), a problem probably related to the prescription habits of doctors. This problem was also reported by Seah et al. (2017), who attributed it to false beliefs related to the fact that broad-spectrum antimicrobials such as carbapenems, at high doses, are perceived as more effective.

In addition, an inadequate indication of the antimicrobial was identified in correspondence with the antibiogram results, probably associated with the lack of availability of drugs. Finally, concerning the problems identified in the therapeutic regimens, it was caused by the incorrect use of the doses and frequencies of administration. The drugs involved were ceftriaxone, where only 1 g was used in the surgical prophylaxis of head and neck surgeries, when 2 g should have been used, considering that these are surgeries with extended performance time (Vilar-Compte et al., 2011). Amikacin was used every 8 hours when it should be used every 12 hours and, in some cases, a single dose is used per day. The use of cotrimoxazole was also detected every 8 hours instead of every 12 hours (MINSAP, 2014).

Considering the individualization of treatment, no dose adjustment was made when using aminoglycosides in elderly patients, nor was it done when using cotrimoxazole in some patients with renal failure. In this regard, the literature refers to the continuous alteration that cancer patients experience in terms of their renal function, especially if they have a nephrotoxic history and if they are receiving drugs that increase the toxicity caused by aminoglycosides. Therefore, it is essential to measure serum creatinine immediately before, during and after treatment with aminoglycosides (Rubio Hernández et al., 2001).

Cancer patients constitute a group predisposed to acquiring intrahospital infections, mainly due to high immunological depression. The use of aminoglycosides should be more cautious, so as not to incur in iatrogenic due to medications that lead to a worsening of the life quality of these patients (Rubio Hernández et al., 2001). On the other hand, the association between the decrease in the glomerular filtration rate in geriatric patients and the risk of toxicity of drugs eliminated via the kidney is well documented. If we also consider the chronicity of the pathologies, the high number of prescribed medications, and the numerous possibilities of pharmacological interactions, the need for an appropriate dosage adjustment to the patient's characteristics becomes evident (Gilbert et al., 2013).

The few drug combinations detected could be attributed to instabilities in the drug supply. Pérez-Martínez et al. (2014) also reported the lack of different antimicrobials availability in their study. Although there is an antimicrobial policy, a drug-therapeutic committee and an antibiotics committee in the hospital institution, there are still shortcomings in the use of antimicrobials such as those described in this study. Therefore, it is necessary to design programs for monitoring the use of antimicrobials at the institutional level, as well as updating institutional

protocols for the use of antimicrobials with a view to contributing to the reduction of inappropriate use of antimicrobials and prevention of microbial resistance.

CONCLUSION

It can be summarized that the inappropriate use of antimicrobials constitutes a problem in cancer patients and highlights the need to implement actions that contribute to raising the quality of prescriptions and the prevention of microbial resistance.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	García DF	Rodríguez MA	Reyes IH	Araya PG	Garrido G	Alvarez MS	Bermudez IC
Concepts or ideas	x	x	x	x	x	x	x
Design							x
Definition of intellectual content			x				x
Literature search	x		x	x	x	x	x
Experimental studies	x	x					x
Data acquisition	x	x					
Data analysis	x	x	x	x	x		x
Statistical analysis	x	x					x
Manuscript preparation	x	x	x			x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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